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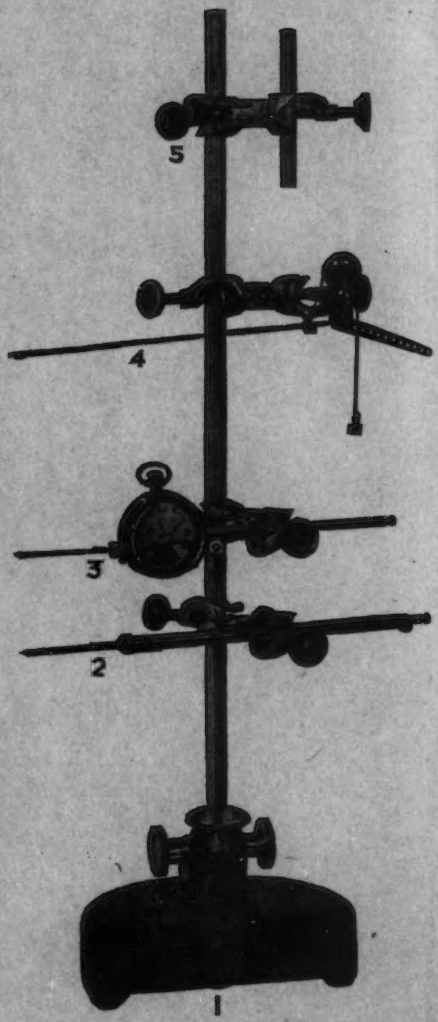

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GASTRIC MOTILITY IN VITAMIN B DEFICIENCY IN THE DOG

ERMA A. SMITH

From the Hull Physiological Laboratory of the University of Chicago

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The literature on gastric motility in the dog during vitamin B deficiency has been recently reviewed by Cowgill (1), hence it will be omitted in this report.

Experimental. Diet I consisted of lean meat, 1 per cent, white bread 4 per cent, and bone ash 0.5 per cent (by weight) mixed together, moistened and made distinctly alkaline with Na_2CO_3. This mixture was autoclaved at fifteen pounds pressure for three hours. Vitamin B is thought to be destroyed by heat in an alkaline medium. Diet I is eaten and relished by the dog for a comparatively long period (100 days or more). After seventy days on diet I the dogs were placed on the vitamin B deficient diet devised by Karr (2). This will be designated diet II. Diet II brings on deficiency symptoms much more quickly (30 to 40 days).

Gastric motility was studied by Carlson's balloon method. The dogs were trained to swallow the balloon and to lie quietly on a table for two to three hours during which time a tracing was taken. Typical records appear in figure 1.

A series of x-ray observations was made of the emptying time of the stomach after a standard barium meal. There seems to be some delay in emptying time after seventy days on diet I, but not enough readings were taken to make the findings conclusive.

Blood calcium determinations upon dogs in spastic paralysis from vitamin B deficient diets ranged from 7.25 to 7.45 mgm. per 100 cc. This is near the tetany level. Electrocardiograms from one of the dogs in spastic paralysis showed consistently an inverted T-wave. This would seem to indicate degeneration in the Keith-Flack node. The heart rate was about 180 per minute.

Golgi preparations of the spinal cord from one of the spastic animals showed marked degeneration in the dorsal columns and some degeneration in the ventral columns. The sciatic nerve showed extensive degeneration.

Discussion. The records from the empty stomach by the balloon method show very active gastric motility during loss of appetite for the vitamine B deficient diet. Periods of hunger peristalsis merge into less active states, followed by periods of quiescence. Tonus becomes lower toward the quiescent period and may approach zero. The same observations have been made upon control animals.

Dogs lose their appetite for a vitamin B deficient diet but retain a voracious appetite for other foods, hence cannot be said to have developed

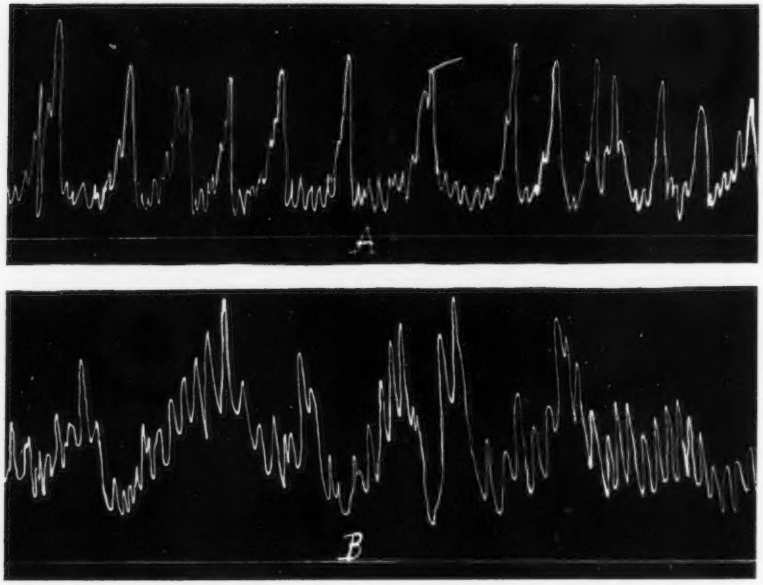

Fig. 1. *A*—Normal dog, typical gastric hunger contractions 48 hours after feeding. *B*—Same animal after 100 days on vitamin B deficient diet (70 days on diet I and 30 days on diet II). Record taken on 4th day after loss of appetite for vitamin B deficient diet.

complete anorexia. During this period of pseudo-anorexia there is active peristalsis of the empty stomach, records of which cannot be distinguished from those of control animals. These facts are not in accord with the interpretation made by Cowgill (1) after similar studies.

When extreme spasticity develops there is gastro-intestinal disturbance and vomiting. If the dog is forced to swallow the vitamin B deficient food violent vomiting follows. It is not possible to keep a balloon in the stomach of the dog when in this condition, hence no satisfactory records

could be obtained. Carlson (3) has reported similar persistent vomiting, accompanied by gastro-intestinal atony and stasis, in extreme parathyroid tetany in dogs, the gut disturbance being apparently a sequence of the extreme nervous disorders.

Dogs fed on diet I for 100 days or more develop extreme cachexia and cannot be restored by placing normal food at their disposal.

CONCLUSION

Dogs which have lost their appetite for vitamin B deficient diets and showing initial symptoms of beri-beri have normal types of gastric hunger peristalsis. This is true after the animal has refused the deficient diet for a period of four days.

I wish to thank Dr. A. J. Carlson for helpful criticism, Mr. Londus Brannon who made the calcium determinations, Mr. Harold Voris who made the histological preparations and Doctor Darrow who took the electrocardiagrams.

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(3) Carlson, A. J. This Journal, 1913, xxxii, 398.

THE SITE OF ACTION OF VERATRINE ON SKELETAL MUSCLE

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From the Rudolph Spreckels Physiological Laboratory of the University of California

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The peculiar action of veratrine is of interest because of its possible relation to "tonus" and a hypothetical double contractile mechanism in striated muscle (Bottazzi, 1901; Ioteyko, 1902). Recently, de Boer (1925), assuming that this drug acts directly upon the muscle fibers, has used it in the study of a plurisegmentally innervated muscle to differentiate the muscle fibers innervated by each segment. As Katz (1925) suggests, the value of de Boer's evidence depends upon whether the site of action of veratrine is upon the muscle fibers or upon the nervous elements. Thus the exact location of the action of veratrine appears of some importance.

Although it seems generally accepted that veratrine acts directly upon the striated muscle fibers, Wible (1924) describes experiments which cause him to conclude that veratrine acts upon the nerve fibers to produce the characteristic contraction in the muscle units innervated by them. Ioteyko (1902) cites similar results but draws the conclusion that the veratrine travels along the nerve fibers to reach the muscle at which point it produces its characteristic action. To determine whether veratrine has an action upon nerve fibers, we have repeated certain of Wible's and Ioteyko's experiments, with some modifications of technique.

Experiments on isolated nerve-muscle preparations. In our experiments, two sciatic gastrocnemius nerve muscle preparations from the same frog were used, one serving as a control. The nerves were held in a specially constructed nerve holder of paraffined wood, as shown in figure 1. The nerves passed through wells (*A* and *B*, fig. 1) 11 mm. in diameter. Outside the wells the nerves passed through slots 1.5 mm. wide carefully packed with petrolatum. The length of this slot between the well and the muscle was 12 mm. Electrodes for stimulation were provided at *C* and *D* (fig. 1). The muscles were suspended in separate baths of Ringer's solution. The knee joint of each nerve-muscle preparation was supported by a thread from a muscle lever, and the Achilles tendon fastened by a thread to a cork at the bottom of the bath of Ringer's solution. Stimulation was provided by maximal break shocks from an inductorium, through the electrodes *C* and *D*. One of the wells, *A*, was filled with a solution of veratrine, the other

well (*B*, control) with Ringer's solution. In a part of the experiments a solution of veratrine sulphate (Powers, Weightman, Rosengarten) in Ringer's solution was used, in the remainder of the experiments a solution of veratrine (U. S. P. Merck). In the latter case, the solution was made acid with hydrochloric acid in order to dissolve the alkaloid and was then brought to pH 7.4 to 7.6 by the addition of sodium bicarbonate. No difference in the action of these two solutions was noted. Wible does not state that a control nerve-muscle preparation was used in his experiments or that the muscle was suspended in salt solution. He supported the nerve on a glass nerve holder, a vaseline ring being placed around the nerve to prevent the veratrine reaching the muscle. Ioteyko applied a strong solution of veratrine to the nerve on a piece of cotton. Both authors describe the appearance of the veratrine contraction upon stimulation of the nerve or the muscle directly, after varying periods of time. They also describe the appearance of spontaneous twitching of the muscles after a certain length of time. In our earliest experiments we sometimes obtained the veratrine contraction upon stimulation after several minutes. However, these results were not constant, and after carefully perfecting our technique in packing the petrolatum about the nerve our results became constantly negative. The muscles were stimulated at intervals of five minutes for as much as one hour after application of veratrine to the nerve. We have seldom seen spontaneous twitching of the muscles in our experiments except where the frogs were infected with "red leg" and the control muscle also showed such spontaneous movements. It seems evident, therefore, that the results of Wible and Ioteyko must have been caused by the leakage of the veratrine from the nerve on to the muscle, but not by passage through the nerve as the latter author concluded, since in most of our experiments the veratrine contraction never appeared. The strength of solution used by Ioteyko is not stated, that used by Wible was approximately 0.074 per cent. We used dilutions of 0.075 per cent, 0.1 per cent and 0.2 per cent with similar results in all cases.

Wible offers as proof that veratrine acts on the nerve only an experiment in which after the appearance of the veratrine contraction the nerve is cut between the point of application of veratrine and the muscle. He states that in this case the veratrine contraction disappears, but the figure which he gives to illustrate this phenomenon is not conclusive. In another paper, Wible (1925) states that the veratrine contraction also disappears when an ethyl chloride block is established between the point of application of the veratrine and the muscle. We are unable to offer an explanation for this result in the light of our own experiments.

We have observed that the irritability of the nerve is reduced by veratrine. The time required to render the nerve inexcitable varies inversely with the strength of the solution, a 0.2 per cent solution requiring about

20 minutes, a 0.075 per cent solution requiring approximately 50 minutes. The veratrine sulphate and veratrine (U. S. P.) solutions gave like results. Boehm (1913) reports results with veratrine (cevadin) corresponding qualitatively with our own.

Experiments on whole animals. As conclusive evidence in favor of her hypothesis that veratrine travels in the nerve, Ioteyko devised the following experiment; the technique is essentially that of the classical curare experiment of Claude Bernard. A frog was pithed and the thigh of one side completely resected with the exception of the sciatic nerve. Veratrine was then injected and after a certain period she found that the veratrine contraction appeared in the gastrocnemius of the side in which the circu-

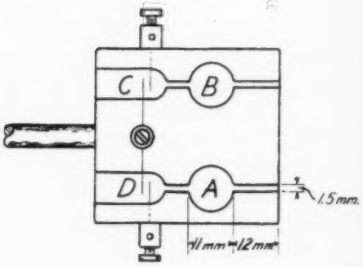

Fig. 1

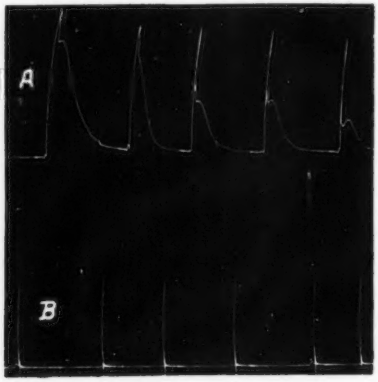

Fig. 2

Fig. 1. Nerve holder. *A*, *B*, wells for solution. *C*, *D*, platinum electrodes.

Fig. 2. Contractions of gastrocnemii of frog 1½ hours after injection of 1 cc. 0.075 per cent veratrine solution. *A*, muscle with circulation intact. *B*, muscle without circulation.

lation was destroyed by the resection, as well as in that of the normal limb. We have repeated this experiment but instead of resecting the thigh we exposed the sciatic nerve and passed a mass ligature around the whole thigh exclusive of the nerve. The brain was pithed above the medulla only, before this operation, in order to insure circulation of the veratrine after injection. Doses of 0.5 cc. and 1.0 cc. of 0.075 per cent veratrine sulphate or veratrine (U. S. P.) were injected into the ventral lymph sac. Within a very short time the muscles of the entire body except those of the ligated leg, exhibited severe prolonged contractions. After this had occurred and the distribution of the veratrine was assured, the spinal cord was pithed to abolish reflexes which might obscure the picture. After periods varying from one-half to one and one-half hours, the frogs were

mounted upon a frog board, the gastrocnemius muscles exposed and the Achilles tendons fastened to muscle levers. The sciatic nerves were exposed for stimulation. Care was taken to use freshly washed instruments in dissecting out the gastrocnemius muscles of the ligated side, in order to assure that no veratrine was carried to them. It was found necessary to keep the frogs horizontal between the time of injection and of mounting in order to prevent serum containing veratrine from running onto the muscle. In these experiments there occurred in the muscle with intact circulation a marked veratrine contraction, upon stimulation. In the muscle without circulation the contraction was normal. Figure 2 shows a record of such an experiment. In some cases a very slight prolonged contraction of the muscle of the ligated side occurred. This was always very much less than that seen in the muscle with circulation intact, and in muscles immersed in very dilute veratrine solution (0.0002 per cent). It is believed that this may have been due to a very small quantity of veratrine having been carried to the muscle by diffusion, or possibly is only a manifestation of the "red leg" with which some of our frogs were infected.

In these experiments, the nerve of the ligated leg is exposed to the action of the veratrine, and since no veratrine contraction (or a negligible amount) occurs in the muscle innervated by it, we believe this drug does not stimulate the nerve fiber as Wible concludes. The length of time elapsed without the appearance of the characteristic effects in the muscle also indicates that the veratrine does not pass through the nerve substance as Ioteyko suggests.

Experiments on the frog's sartorius. It is conceivable that veratrine may act upon the myo-neural junction rather than upon the muscle fibers themselves. Wible (1924) describes experiments on the frog's sartorius in which he states that if the muscle be sectioned transversely through the middle, the lower half which is presumably devoid of nervous elements does not show the veratrine contraction upon stimulation. We have repeated this experiment with somewhat varying results, which however do not tend to confirm this statement. Prevost in 1867 (as stated by Sollmann, 1924) showed that veratrine affects the nerve-free end of the sartorius and also that the veratrine contraction occurs after curarization. Bottazzi (1901) produced curarization in one gastrocnemius of frogs but not in the other, by ligature of one leg before the injection of curare. He then removed the gastrocnemii and treated them with veratrine, but could find no difference in the veratrine curve of the two muscles. Certain of our experiments on the sartorius were performed upon muscles from curarized frogs with no apparent alterations in the reaction to veratrine. It thus appears that the site of action of veratrine is peripheral to that of curare and, therefore, probably in the muscle fiber itself.

CONCLUSIONS

1. Veratrine destroys the excitability of the nerve fibers without stimulating them or producing the characteristic veratrine contraction upon artificial stimulation.

2. Veratrine does not travel through the substance of the nerve.

3. The site of action of veratrine on skeletal muscle is peripheral to that of curare, probably the muscle fiber itself.

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PERISTALSIS IN THE DOGFISH AND RAY

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During all the years that I have struggled with the difficulties which interfere with the study of peristalsis, the paralysis which ensues when the abdomen is opened, and the impossibility of recording simultaneously the contractions of many parts of a long winding tube, I have wished for a simple lowly animal with a short easily-accessible intestine and a nervous system so hardy that its abdomen could be opened without shock. It was with this thought in mind that I went to Woods Hole, and there I was rewarded by finding that, to a large extent, the dogfish and ray could supply my desiderata. The studies here reported were not undertaken with any idea of dealing adequately with the subject of digestion in these animals, if only because the time at my disposal was too short. What I did hope to do was to spy out the land, and to see how promising it might be for future more extended explorations.

TECHNIC. The first animal to be studied was the dogfish (*Mustelus canis*), a small shark varying in length from perhaps 40 to 80 cm. The anterior part of the brain was severed from the medulla by thrusting a knife through the cartilaginous skull along a line connecting the posterior canthi of the eyes. The animals were then placed on the back in a trough and sea water was allowed to run over the head. Only occasionally was there any difficulty with respiration. The abdomen was opened and the sides held upward and outward with threads. The viscera were kept moist either with sea water or with Ringer's solution made up according to Mines' formula, with 2 per cent of urea (Mines, 1912) (Athias, 1920). Both seemed to be equally efficacious. The left lobe of the liver was drawn forward out of the abdomen.

ANATOMY OF THE STOMACH. As in most fishes, there is not much sign of a sphincter at the cardia, but there is a marked difference there in the appearance of the blood vessels. They run longitudinally on the esophagus and transversely on the stomach. As compared with the rest of the

[1] I wish here to express my appreciation of the kindness of Dr. Frank R. Lillie and Dr. M. H. Jacobs who placed every facility of the Marine Biological Laboratory at my disposal.

digestive tract, the stomach is large; it looks much like that of a carnivore, and in a large dogfish is about the size of that in a small dog. The main pouch ends in what appears to be a small muscular cecum which from time to time has a tendency to contract into a hard white knot. In that region, corresponding probably to the incisura on the stomach in man, the blood vessels running along the lesser curvature go straight on to anastomose with those on the greater curvature. They thus produce a picture much like that at the pylorus in man where, as W. J. Mayo showed years ago, the presence of a transverse vein serves to identify the juncture between stomach and bowel.

Parallelling the lesser curvature and fastened to it is a narrow tube from 10 to 15 mm. in diameter and from 5 to 15 cm. long, depending on the size of the dogfish. Its color is usually redder than that of the stomach, due apparently to a better blood supply, but occasionally in flabby animals it has a bluish tint. There is often a marked transition in the color of the tissues at the line joining this narrow bowel-like tube and the large gastric pouch, a change which adds to the impression that the pylorus is situated at this point. The anatomists are doubtless right, however, in calling this tube a part of the stomach because the ray has an organ which is identical except that there is no sign of a pylorus at the upper end. Moreover, in both the dogfish and ray there is a definite pylorus at the lower end where the tube joins the valve-gut. At this point, again, there is marked transition in color, a difference which is most striking when, as often happens, the pars pylorica above contracts until it becomes white and the intestine below remains red and full of blood. I have remarked elsewhere on a similar block between the muscle fibers on either side of the pylorus in man, due apparently in large measure to the separation of the muscle bundles at this point by many bands of connective tissue (Alvarez, 1922). A study of sections from this region in the dogfish shows a similar breaking up of the muscle into small islands surrounded by connective tissue. Sections made from the juncture of fundus and pars pylorica showed much the same picture which, again, probably accounts for the blockage of the waves in this region.

The interesting point about all this is that in the laboratory animals the body and the pars pylorica of the stomach often act as if they were two organs. In the dogfish they not only act like two organs but they look different. In man a stoppage of the waves at the upper end of the pars pylorica is seldom seen; in laboratory animals it is quite common; and in the dogfish it seems to be almost the rule. Figure 1 *B* shows the lack of correlation between the movements in the two parts. Only occasionally did I see a wave which seemed to travel from the cardia all the way to the pylorus.

It is interesting to note also that, in the earliest stages of the embryo-

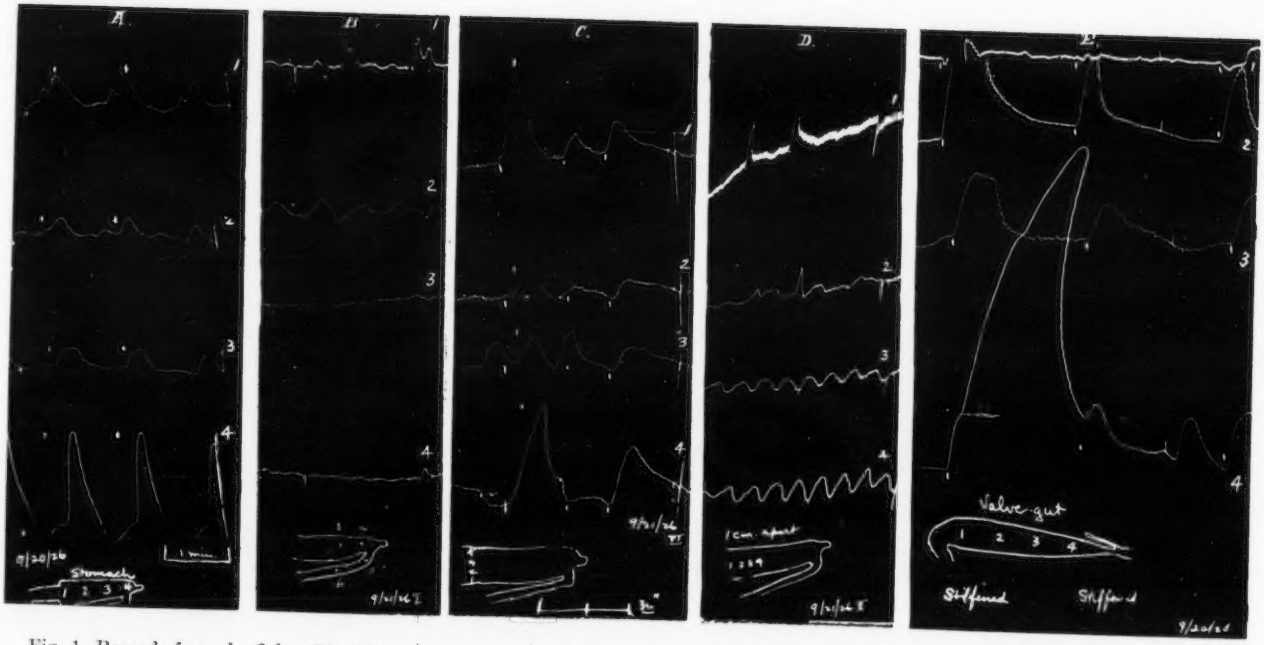

Fig. 1. Records from dogfish. The diagrams show the location of the recorders on the stomach and valve-gut. The records read from left to right. The magnification of the levers was about 7 times. Synchronous points are marked. All records were made with about the same slow speed of drum. *A* shows peristalsis in the main gastric pouch; *B* shows the independence of the two parts of the stomach; *C* shows synchronous and dissociated contractions at the cardia; *D* shows a downward shifting of the pacemaker, and *E* shows systolic stiffenings of the valve-gut.

logic development of the stomach in man, the pars pylorica is indistinguishable from the intestine, and a small cecum develops at about the same point as in the dogfish. In man this cecum grows so fast that in the adult it constitutes most of the stomach (Lewis, 1912). In the dogfish, as in man, the secretion of the pars pylorica is alkaline and contains no digestive ferments (Sullivan, 1905).

Gastric peristalsis. In many ways the peristaltic activity of the stomach of the dogfish resembles that in man. When the waves are coursing regularly and deeply they appear two or three times a minute somewhere near the cardia, and travel in sets of two or three downward over the main pouch. Graphic records made by fastening threads to various parts of the stomach showed that, in this fish, as in the usual laboratory animals, the stomach and bowel are never quite at rest. When waves are apparent to the naked eye they are generally peristaltic in character; when they are invisible without the help of magnifying levers the records show small contractions here and there which seem to be poorly coördinated and hard to interpret. These records show also that besides the peristaltic waves there are slow tonus changes and small contractions with fairly rapid rhythm (Alvarez and Zimmermann, 1926).

In an attempt to learn something about the pacemaker, I attached four recording threads along a line parallel to the esophagogastric juncture and 1.5 cm. caudad to it. The records obtained show that when strong waves were running over the stomach, all parts on the ventral surface of the cardia were contracting at about the same time, but that when the gastric waves were shallow and poorly coördinated the pacemaker was shifting about (fig. 1 *C*). Several times it seemed to be located in an area about 4 cm. caudad to the cardia (fig. 1 *D*).

Sometimes the outer side of the cardia would contract first, and the wave would spread slowly to the inner side before going on down the stomach. Occasionally this would seem to produce a circus movement at the cardia which, as in the auricle of the heart, would either fail to send a wave off caudad or would give rise to a wave with two crests. This was a helpful observation because extra crests are so common in records of the rabbit's stomach. In the dogfish, however, it could be seen that these double crests can arise in another way. A deep wave would be seen traveling downward over the stomach when suddenly another would appear 1 or 2 cm. ahead of it and go forward, maintaining that distance. In that case the new wave seemed to arise in the muscle which had been pulled upon and stimulated by the advancing ring of contraction. When these new contractions appeared at the pylorus or incisura they tended to spread backward to block the main descending wave, just as they do in the higher animals and in man.

One great difficulty in interpreting the graphic records arises from the

fact that when contraction begins at the cardia the gastric wall for some distance below is pulled on and stretched, and the recorders are affected. For this reason the contractions in different parts of the stomach may all seem to start at the same time, and the progressive nature of the process can be seen only by studying the crests.

CONTRACTIONS IN THE PARS PYLORICA. As has already been mentioned, the waves on the main gastric pouch rarely went on down the tubular portion, and at no time were deep forwarding contractions seen. Ordinarily, blanching contractions appeared on the pars pylorica on the side of the greater curvature near the gastric cecum, and traveled slowly caudad. Relaxation ahead of these waves would probably have shown itself by an opening up of the blood vessels and a reddening of the tissues, but this was never observed. Occasionally the diameter of the tube ahead of the advancing wave seemed to increase slightly, but that was due to the shortening brought about by the contraction of the longitudinal muscle. Just as in the upper gastric pouch, the advancing trough pulled on the tissues for some distance ahead.

On one occasion there was seen advancing over the pars pylorica a ring of contraction which did not obliterate the blood vessels. Apparently it was traveling in the circular muscle alone. This was of interest because in the higher animals the two layers of muscle supposedly contract together. Still more striking, instances of this dissociated activity were observed when the spindle-shaped valve-gut would suddenly stiffen and almost double in length. The graphic records showed that this movement was caused by a contraction of the circular muscle with, at the same time, a lengthening of the longitudinal (fig. 1 *E*).

When the pars pylorica was stimulated by the prick of a pin the muscle was pulled in from all sides. If the stimulus was slight or the animal insensitive, only a tiny white wheal resulted, but if the stimulus was strong a white trough appeared which ran transversely around the bowel, producing raised edges above and below. If the stimulus was applied at the upper end of the tube this trough traveled caudad; if it was applied near the pylorus it might travel orad. It was interesting to see a pink area of relaxation sometimes traveling caudad over a region which had contracted until it was white.

VOMITING. Just as in the higher animals, so in the dogfish, attaching serrefines to the stomach or handling it often induced gasping and struggling. On one occasion, while I was handling the stomach and pulling on it, the animal suddenly began to retch, and soon vomited some half-digested fish. This vomiting seemed to be effected by contractions of muscles in the thoracic region, because the abdomen was open from end to end and I did not feel any contraction of the stomach which was in my hand. Immediately after the vomiting a strong contraction appeared about the

middle of the stomach and spread both ways. A powerful wave began at the pylorus also and spread slowly backward to the main gastric pouch, emptying all the contents of this region before it. Retching was induced several times more by pulling on the esophagus, or by sticking the handle of a scalpel into the pharynx. Later the center became insensitive and the reflex could no longer be obtained. The contraction which spread up the pars pylorica stopped at the main gastric pouch. When the animal was retching the stomach did not contract sufficiently to empty itself.

THE VALVE-GUT. Distal to the pylorus one finds the large cigar-shaped section of a bowel which contains the spiral valve. This valve begins at the tip of a conical projection from the pylorus and runs like a circular stairway almost to the cloaca. As Parker has shown, it serves to increase greatly the absorbing surface and to make up for the short length of the bowel (Parker, 1880). At the lower end of this spindle-shaped gut where it joins the cloaca there is a small finger-like organ, the so-called rectal or digitiform gland, which seems to correspond to the appendix of higher animals (Howes, 1890) (Pixell, 1908). If this be so, the valve-gut must take the place of the whole of the small intestine and these fishes must have no colon.

In most of the animals the valve-gut did not show much activity. In some a wave-like contraction began at the cloacal end and spread upward, pulling the large mesenteric vessels to the left. From the tail end of the fish the rotation of the wall was clockwise. There were about two and a half of these waves a minute, and they took about twenty seconds to travel up the bowel. On one occasion the graphic record showed ten small waves a minute. In another animal the lower valve-gut showed something like rhythmic segmentation, associated with reverse waves which faded out after ascending a short distance. As already mentioned, this part of the digestive tract would occasionally stiffen and almost double in length. More rarely a similar lengthening and stiffening of the stomach was observed. This is an interesting phenomenon which I do not remember seeing in the higher animals unless perhaps it takes place slowly during the production of intussusceptions. On one occasion there was also a systolic shortening of the valve-gut.

The most interesting movement of the valve-gut is a sudden outward (counter-clockwise) rotation of the whole organ. This is due to the contraction of a remarkable sheet of smooth muscle in the mesentery, which is attached only at the upper end of the bowel. As Cannon remarked when he discovered it in 1907, it would seem to be particularly suitable for studies on the physiology of smooth muscle (Cannon, 1908). In one animal I cut it away from the bowel, fastened a heart lever to it and obtained records of rhythmic contractions at the rate of 2.6 each minute. In both the dogfish and ray this sheet of muscle seemed to be the most sensitive structure

in the abdomen, as the slightest pull or pinch would cause the animals to gasp or struggle. The same stimulus applied to other parts of the peritoneum had no such effect.

THE RAY (*raia erinacea*). Studies were made on two rays. The digestive organs of this species are almost the same as those of the dogfish. Deep peristaltic waves were occasionally seen on the main gastric pouch, and on one occasion one traveled all the way from the cardia to the middle of the valve-gut. At other times reverse waves were seen in different parts of the stomach. Once a series of them was seen running over the pars pylorica, and occasionally a normal downward wave was so reflected at the pylorus as to run backward a few centimeters. Sudden stiffening movements of the stomach were seen from time to time.

Graphic records of the cardia showed small rhythmic contractions at the rate of about ten a minute. Waves with two crests spreading over the stomach were due sometimes to two regions of the cardia contracting, one a little ahead of the other. Powerful contractions appearing at the cardia did not always spread over the stomach. No movements were seen in the valve-gut.

PERISTALSIS IN STRIATED MUSCLE. As some men have the impression that peristalsis is associated only with smooth muscle and a nervous plexus, it was interesting in one of the dogfishes to watch for an hour or more peristaltic waves running regularly over a band of striated muscle situated under the skin of the tail. On my return to Rochester Doctor Robertson kindly looked at sections from this region and found, mixed in with the skeletal muscle, one narrow band which may have been made up of smooth muscle or some primitive form of connective tissue. It is conceivable that this small bundle might have originated pulsations, but it certainly was not big enough to produce the waves which I saw. These waves appeared every two seconds near the tip of the tail on the left side near the dorsum and ran forward for about 6 cm. The rate of conduction was so rapid that one wave finished before another began. The distance between troughs was about 8 mm., so that there was no resemblance to the larger undulatory swimming movements. They were more like the waves which I have often seen in the pectoral muscles of emaciated men and women. If one strikes such muscles with the finger or a hammer, a little wheal forms with its long axis perpendicular to the direction of the fibers, and two other small wheals ripple outward for a few centimeters, one toward the sternum and the other toward the axilla.

DISCUSSION. Although in some ways the dogfish is hardy and immune to shock, in other ways it is not so hardy as the other fishes caught in the waters around Woods Hole. It is the first to lose strength and die when kept in a large wire-net tank immersed under a float in the harbor, so it is probably best, especially with the larger fishes, to use them within twenty-

four hours of the time they are caught. The ray seemed somewhat more tolerant of the conditions in the tanks.

It was disappointing to find the same baffling shallowness of peristalsis in four out of five of these fishes, as in four out of five rabbits or cats; and again, it was impossible to say why one animal had good deep waves for hours at a time while the others had almost none. The experience with these dogfishes and with men and women examined under the roentgenoscope makes me feel now that our difficulties and disappointments with animals opened under salt solution are not all due to inhibition but to some individual peculiarity. In many cases the cause may be found in the fact that the animal is sickly or below par, but many exceptions to that rule can be found, and in men and women the most tumultuous peristalsis is sometimes seen in the weak and ailing.

A nice feature about working with fishes is that movement is slower in them that in warm-blooded animals; it is like studying action with the slow-motion camera and one can be surer of what is taking place. There are, however, some exceptions to this statement and at times it is surprising to note the quickness with which this cold-blooded muscle can contract.

The tissues are quite translucent, so that with a binocular loupe one can see the delicate blood vessels and can trace the progress of a peristaltic wave either by the approximation of these vessels or by their obliteration. As I watched them moving like white clouds over a pink sky, I was impressed with the need for understanding why they begin and why they fade; why they sometimes produce a deep cramp-like contraction and at other times a shallow ineffective one; why at times the contraction travels and at others it does not; why it generally travels caudad and why it stops so sharply at the sphincters; why at times it goes from one end of the tract to the other, and why at others it fades out after going a short distance.

In the dogfish the contraction seems generally to spread from muscle fiber to muscle fiber, perhaps along the bridges of a syncytium. The shortening of the fibers at one spot stretches neighboring fibers and that also serves as a stimulus for the production of a wave-like contraction. The sudden lengthening of the stomach and bowel must be brought about by the simultaneous, or almost simultaneous, contraction of the whole circular muscle sheet, in which case the stimulus almost certainly must travel through the nervous plexus. There may also be a reciprocal relaxation of the longitudinal coat. The puzzle then is: Why does a powerful contraction spread sometimes only from fiber to fiber, and why at other times does a slight touch spring the trigger of the nervous mechanism so as to produce a sudden systole?

SUMMARY

The mechanics of digestion can be studied to advantage in selachians, as they are easily handled, easily decerebrated, and quite tolerant of operative procedures. The whole digestive tract can be seen at a glance; peristalsis is slower than in higher animals, and the waves can easily be followed as they travel over the translucent tissues.

The stomach of the dogfish somewhat resembles that of the human embryo. Peristalsis is similar to that in higher animals except that there is less correlation between the movements in the fundus and the pars pylorica. When the stomach is quiet the waves often seem poorly coördinated and the pacemaker shifts about. Complete dissociation was observed at times between the activities of the circular and longitudinal muscles. The dogfish can vomit. Retching movements are associated with reverse peristalsis in the stomach. Reverse peristalsis appears to be normal in the valve-gut. The organ at times stiffens suddenly and lengthens out. It also is rotated outward by a peculiar sheet of smooth muscle constituting the mesentery. Peristaltic waves were seen traveling over the striated muscle in the tail of the dogfish.

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A STUDY OF THE COAGULATION OF THE BLOOD; THE CHEMICAL REACTIONS UNDERLYING THE PROCESS

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The coagulation of the blood has been very generally regarded simply as a protective mechanism which prevents undue loss of blood as a result of injury to the walls of the blood vessels during life; the process involves reactions between substances, namely, protein and lipoid, which have come to be looked upon as intimately associated with the cellular processes of nutrition and immunity. It has long been recognized in human pathology that a relationship exists between infection and thrombosis, which very commonly develops during the immunization of both horses and rabbits by the intravenous injection of virulent pneumococci. The destruction of the pneumococcus by lysis in the pneumonic exudate has long been known, and, more than twenty years ago in the course of a study of pneumonia (1), it was noted that this destruction occurred early, apparently during fibrinogenesis, which, since the pneumococcus ordinarily is not susceptible to lysis, suggested the possibility of a direct relation between the phenomenon of bacteriolysis and the coagulative process. The relationship of anaphylaxis to the adaptative process of immunity is established, and well-marked changes in the coagulability of the blood are associated with shock. Additional evidence of the possibility of an interrelationship between these phenomena may be found to exist in a very striking observation which was made in the course of a study of the action of immune serum in pneumococcus infection: typical anaphylactic shock occurred ten to thirty hours after the intravenous injection of immune serum into rabbits which had previously been inoculated intravenously with virulent pneumococci. In 1921, a direct relationship between the coagulative process and the phenomenon of conglutination (2), and also the agglutination and hemolysis (3) of sheep cells by normal bovine serums was found to exist. These observations, together with other related facts in the literature, suggested the study of the coagulation of the blood, the results of which it is the purpose of this paper to record.

PREVIOUS WORK ON THE COAGULATION OF THE BLOOD. The fact that the

coagulation of the blood is due to the formation of an insoluble substance, fibrin, derived from its soluble antecedent, fibrinogen, was perhaps first postulated by Virchow (4). Despite all subsequent study during the past century, the fundamental reactions of fibrinogenesis are still quite obscure. Modern concepts of thrombin as the active agent date from the observations of Buchanan (5) in 1845 and were more fully developed sixteen years later by Schmidt (6) (7) (8) (9) (10), but it was Wooldridge (11) who, in 1893, studied the chemical nature of the process and demonstrated the importance of a lipoid, lecithin. That a definite concentration of free calcium ions is also essential was recognized in the early work of Arthus and Pages (12) and later by Sabbatani (13). Similarly, the influence of contact with a strange surface was demonstrated by Freund (14) as early as 1883. It is not within the scope of this paper to discuss the theoretical conceptions of these and other workers; nor is it necessary here to review in further detail the observations contained in a long list of investigations upon which are based present-day theories; more particularly, the studies of Hammarsten (15) (16) (17), Pekelharing (18), Morawitz (19), Fuld and Spiro (20), Nolf (21) (22) (23) and Bordet (24) (25) (26) (27). Special attention has been devoted to the lipoids in the work of Bordet and his collaborators, of Zak (28), Gratia and Levene (29), Mills and Mathews (30) (31) but it was Howell (32) who first recognized the cephalin fraction of the lipoid as the active agent. Coagulation has also been studied from the point of view of the action of inhibitory substances and Howell's studies (33) (34) in this field led to his discovery of heparin. The present study does not deal with this phase of coagulation but has been devoted almost exclusively to the investigation of the chemical reactions of the active processes following the experimental methods of Bordet which seemed to offer the more promising and direct approach.

PURPOSE AND SCOPE OF THE PRESENT WORK. Prior to the work of Bordet and Delange, all of the so-called "thrombin solutions" were derived from blood, or plasma, subsequent to coagulation. These authors, however, obtained active solutions, derived from plasma before coagulation had intervened, which they believed to be free from fibrinogen.

The present work started with an investigation of these solutions together with the plasma from which they had been derived, to ascertain whether any new relationships could be discovered which would alter the significance of the facts previously observed. The results of this study led to an investigation of the chemical reactions involved and the chemistry of the substances concerned in them.

Experiments on the activity of proferment. In their most ingenious experiments, Bordet and Delange (27) appear to dissect plasma, deriving therefrom a proferment which they term serozyme. By following their technic exactly, their observations were confirmed; but their conclusions

were based upon the supposition that they were able to separate a substance, a proferment, free from fibrinogen, which, in the presence of calcium and lipoid extract, acquires the property of transforming fibrinogen into fibrin. This substance, serozyme, was isolated in two ways: by adsorption with gelatinous tricalcium phosphate, and by removal of fibrinogen from a plasma by precipitation with 30 per cent sodium chloride. The experiments seemed to them to give conclusive proof that some substance other than fibrinogen is present in plasma which reacts with, or is activated by, calcium and lipoid extract to produce an active coagulant. This viewpoint was further strengthened by the fact that the supernatant phosphate plasma, after removal of the gelatinous phosphate with its adsorbed substance, was incoagulable by calcium and lipoid extract alone but was rapidly coagulated by the activated proferment. The stability of this phosphate plasma led them to conclude that the treatment with gelatinous tricalcium phosphate removed the proferment completely, leaving behind inert fibrinogen.

One might suppose that such a method of complete isolation of the serozyme would give rise to the most active proferment obtainable from a given plasma.

Experiment 1. Phosphate plasma serozyme, proferment 1, and salt plasma serozyme, proferment 2, were prepared from 10 cc. portions of cell-free oxalated rabbit plasma, as described by Bordet and Delange. The volume of the proferment 2 solution, after dialysis, was 20 cc., corresponding to the total volume of the proferment 1 solution. Calcified salt solution was prepared by adding 0.07 per cent calcium chloride to 0.85 per cent sodium chloride solution. The lipoid extract (cytozyme) suspension was prepared as described by Bordet and Delange.

Protocol 1. Comparison of the activity of salt plasma serozyme and phosphate plasma serozyme of Bordet and Delange

REAGENTS	QUANTITIES			
	cc.	cc.	cc.	cc.
Proferment I, phosphate plasma method	0.3	0.3	0	0
Proferment II, salt plasma method	0	0	0.3	0.3
Lipoid extract (cytozyme)	0.1	0	0.1	0
Calcified salt solution	0.2	0.2	0.2	0.2
Physiological salt solution	0	0.1	0	0.1
Water bath, 37°C., six minutes				
Phosphate plasma	0.1	0.1	0.1	0.1
Clotting time, water bath, 37°C.	4 min.	9 min.	10 sec.	5 min. 30 sec.

The solution of proferment II, remaining after precipitation of the fibrinogen from plasma after treatment with calcium chloride and lipoid extract, clotted phosphate plasma in ten seconds as compared to four

minutes for that of proferment I, obtained by adsorption on tricalcium phosphate. Treatment with calcium chloride alone also showed a comparative difference in the activity of these solutions. In repeating these experiments, moreover, it was found that a uniformly more active serozyme could be obtained from an equal amount of plasma by the salt precipitation method.

Since the solution of proferment II proved more active than that of proferment I, it might be expected that the fibrinogen precipitated in the preparation of the former, after solution in physiological salt solution, would contain less proferment and would be less coagulable in the presence of calcium and lipoid extract than the residual phosphate plasma after removal of the gelatinous tricalcium phosphate.

Experiment 2. The precipitate of fibrinogen obtained in the preparation of pro ferment II from 10 cc. of 0.1 per cent oxalated rabbit plasma was dialyzed for from four to five hours in 0.1 per cent oxalated salt solution. This solution was adjusted to approximate neutrality with dilute sodium hydroxide. Phosphate plasma was prepared from 10 cc. of the oxalated rabbit plasma. The coagulating action of calcified salt solution (containing 0.14 per cent calcium chloride) and lipoid extract (cytozyme) was then determined, as indicated in the following protocol.

Protocol 2. Comparison of the coagulability of salt plasma fibrinogen and phosphate plasma by calcium and lipoid extract

REAGENTS	QUANTITIES							
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Calcified salt solution	0 3	0 3	0	0	0 3	0 3	0	0
Lipoid extract (cytozyme)	0 1	0	0 1	0	0 1	0	0 1	0
Physiological salt solution	0 2	0 3	0 5	0 6	0 2	0 3	0 5	0 6
Salt plasma fibrinogen	0 1	0 1	0 1	0 1	0	0	0	0
Phosphate plasma	0	0	0	0	0 1	0 1	0 1	0 1
Clotting time, water bath, 37°C.	12 min.	15 min.	0*	0*	0†	0†	0†	0†

* No clot in 6 hours; clotted in 20 hours.

† No clot in 20 hours.

As shown in experiment 2, the fibrinogen obtained from oxalated rabbit plasma by precipitation with 30 per cent dry sodium chloride was, after resolution in oxalated salt solution, coagulated by calcium in fifteen minutes, and by calcium and lipoid extract in twelve minutes, while phosphate plasma was not coagulated in twenty hours under the same conditions.

A solution of fibrinogen obtained by precipitation of phosphate plasma with sodium chloride, when treated with calcified salt solution or calcified salt solution and cytozyme, did not form a distinct clot until after twenty-four hours.

The results of these experiments suggested that the phosphate plasma might owe its stability to the development of some inhibiting agent rather than to the removal of a proferment. When gelatinous tricalcium phosphate was added to physiological salt solution in the same proportion as it was added to plasma in the preparation of proferment, the solution became distinctly alkaline. The addition of the phosphate to distilled water, however, did not change its titer. Evidently, the calcium phosphate was decomposed by the sodium chloride, setting free alkali and undoubtedly, also, soluble phosphates.

Experiments on the stability of phosphate plasma. As a result of these observations, the influence of alkali and alkali phosphates upon oxalated plasma was studied to determine whether they possessed an inhibiting effect of a nature to account for the properties of phosphate plasma. The addition of a small amount of sodium hydroxide to oxalated rabbit plasma delayed its coagulation by calcium and lipoid extract; and the addition of secondary sodium phosphate to such an alkalinized plasma, completely inhibited its coagulation by these substances although the effect of the sodium phosphate alone was inappreciable.

Experiment 3. One drop of 10 per cent secondary sodium phosphate was added to 2 cc. of 0.1 per cent oxalated rabbit plasma. Sodium hydroxide N/60, was prepared in physiological salt solution.

Protocol 3. The inhibiting effect of alkali and alkali phosphate upon the coagulability of oxalated plasma by calcium and lipoid extract

REAGENTS	QUANTITIES			
	cc.	cc.	cc.	cc.
N/60 NaOH solution	0	0	0.1	0.1
Calcified salt solution (0.07 per cent)	0.3	0.3	0.3	0.3
Lipoid extract (cytozyme)	0.1	0.1	0.1	0.1
Physiological salt solution	0.1	0	0.1	0
Oxalated rabbit plasma	0.1	0	0.1	0
Plasma + Na_2HPO_4	0	0.1	0	0.1
Clotting time, water bath, 37°C	4 min.	4 min.	5 min.	0

Test observed for 18 hours.

As shown in experiment 3, amounts of alkali and of secondary sodium phosphate which separately had little or no effect upon the coagulation of oxalated rabbit plasma by calcium and cytozyme, together completely inhibited coagulation by these substances. Furthermore, a plasma which has been prepared by the addition of the quantities of sodium hydrate and secondary sodium phosphate used in the above experiment, and which is, therefore, incoagulable by calcium and lipoid extract, becomes coagulable on the addition of an active solution possessing the properties attributed to

thrombin. Thus, it reacted, in every respect, like phosphate plasma although no proferment had been removed.

Experiment 4. One drop of 10 per cent secondary sodium phosphate and 0.033 cc. of N/1 sodium hydroxide were added to 2 cc. of 0.1 per cent oxalated rabbit plasma.

Protocol 4. The coagulation of oxalated rabbit plasma treated with alkali and alkali phosphate

REAGENTS	QUANTITIES	
	cc.	cc.
Proferment II	0.3	0.3
Lipoid extract (cytozyme) suspended in calcified salt solution	0.2	0.2
Physiological salt solution	0.1	0.1
Water bath, 37°C., six minutes		
Phosphate plasma (rabbit)	0.1	0
Oxalated rabbit plasma + sodium phosphate and sodium hydroxide	0	0.1
Clotting time, water bath, 37°C	30 sec.	30 sec.

As indicated in experiments 3 and 4, the coagulating properties of the phosphate plasma of Bordet and Delange do not differ from those of oxalated plasma treated with alkali and alkali phosphates.

It is very probable, in view of these results, that phosphate plasma owes its stability rather to the decomposition of some of the tricalcium phosphate than to the removal of serozyme.

Experiments on the fibrinogen content of proferment solution. It seemed logical to determine at this point whether the proferment—serozyme—solutions were free from fibrinogen, as Bordet and Delange believed. The solution prepared by the method of sodium chloride precipitation was concentrated to a small bulk by pervaporation (35). A portion of this concentrated solution was treated with calcified salt solution and lipoid extract. A well-formed clot of fibrin was produced. The other solution, that is, the proferment from phosphate plasma, was treated in a similar way, but no clot was obtained. It was found, however, that the addition of ammonium sulphate crystals to the solution in a concentration of 30 per cent gave rise to a precipitate. This precipitate, when dissolved in oxalated salt solution and treated with calcified salt solution and lipoid extract, gave rise, after several minutes, to a few shreds which, after slight shaking, were converted into a compact ball of fibrin.

Experiment 5. Salt plasma, proferment II, was prepared from 10 cc. of 0.1 per cent oxalated rabbit plasma. After dialysis, it was concentrated to about 5 cc. in a collodion bag suspended in a current of warm air. It was then redialyzed for

about two hours. (The volume was not affected by this second dialysis.) The solution of proferment I of phosphate plasma was prepared from 10 cc. of oxalated rabbit plasma. The 20 cc. of this solution obtained were treated with 6 grams of ammonium sulphate crystals. This mixture was allowed to stand overnight, and the precipitate formed was removed by centrifugalization. The precipitate was dissolved in 1 cc. of physiological salt solution containing 0.1 per cent sodium oxalate and a trace of sodium hydroxide. These solutions were then tested, as indicated in the following protocol.

Protocol 5. Demonstration of fibrinogen in the concentrated salt plasma and phosphate plasma, proferment or serozyme solutions of Bordet and Delange, and of their coagulating (serozymic) activity for phosphate plasma

REAGENTS	QUANTITIES			
	cc.	cc.	cc.	cc.
Proferment II, concentrated	0.5	0.3	0	0
Proferment I, concentrated	0	0	0.3	0.3
Calcified salt solution	0	0	0.2	0.2
Lipoid extract	0	0	0.1	0.1
Lipoid extract (cytozyme) suspended in calcified salt solution	0.2	0.1	0	0
Physiological salt solution	0	0.2	0	0
Water bath, 37°C.		6 min.		6 min.
Phosphate plasma (rabbit)	0	0.1	0	0.1
Clotting time, water bath, 37°C.	11 min.	3 sec.	6 min. 30 sec.	10 sec.

As shown in experiment 5, both of the concentrated serozyme solutions clotted under the influence of calcium and lipoid extract without the addition of any other source of fibrinogen. The solution of proferment II brought about the coagulation of added phosphate plasma more rapidly than did that of proferment I.

Comparison of the serozyme solutions revealed less activity in the proferment prepared by adsorption on tricalcium phosphate precipitate, which, according to Bordet, removes it completely from plasma, than in the solution left after precipitation of the fibrinogen by 30 per cent sodium chloride. The solution of fibrinogen precipitated with 30 per cent sodium chloride was readily coagulable, whereas the phosphate plasma failed to coagulate in the presence of calcium and lipoid. Moreover, the stability of the phosphate plasma remaining after treatment with gelatinous tricalcium phosphate, is due to the presence of alkali and alkali phosphates. Finally, the two preparations of proferment contained fibrinogen and in quantities approximately proportional to their different degrees of activity. Hence, the assumption that fibrinogen is inert is not substantiated by these experiments which rather suggest that the activity of such solutions depends upon the presence of this substance.

The fact that ionized calcium salts, the protein fibrinogen and a lipoid, supposedly cephalin, were essential to the formation of fibrin in these solutions led to the investigation of the chemical relations existing between these substances.

STUDIES ON THE REACTION BETWEEN IONIZED CALCIUM SALTS AND LIPOIDS. Although calcium and lipoid have been recognized as essential to the reactions concerned in coagulation, no definite chemical combination between calcium and lipoid has been demonstrated. In the course of these experiments on proferment, it was noted that, when the acetone insoluble lipoids or alcoholic tissue extracts were suspended in calcified salt solution, a more turbid emulsion was obtained than when it was suspended in salt solution free from calcium. Moreover, the calcified saline suspension became slightly but distinctly more acid, and, upon treatment with ammonia, a heavy precipitate was formed in the calcified suspension while the saline suspension without calcium remained clear. These reactions suggested that calcium chloride reacted chemically with lipoid, liberating acid.

TABLE 1

Results of the elementary analysis of the calcium-lipoid compounds prepared as described in experiment 6

SAMPLE	RESULTS IN MILLIGRAMS PER 100					
	Carbon	Hydrogen	Total nitrogen	Amino nitrogen	Phosphorus	Calcium
1	57.71	9.03	1.23	0.73	3.91	6.2
2	59.5	9.27	0.92	0.94	4.46	4.95

Preparation of the calcium lipoid compound. Two samples of the calcium lipoid compound were prepared by direct precipitation of alcoholic extract of heart tissue with calcium chloride in ammonia, as described in experiment 6, and were analyzed.

Experiment 6. An alcoholic extract of beef heart tissue was diluted with five volumes of distilled water, made alkaline with ammonia and precipitated with calcium chloride in the proportion of 500 cc. of normal calcium chloride to 7 liters of diluted extract. The slightly brownish precipitate was washed by decantation with alcohol beginning with a 20 per cent concentration and gradually increasing to absolute alcohol. The alcohol was separated from the precipitate by filtration through hard filter paper and, after being dried in the air, the precipitate was redissolved in toluol in which it was almost completely soluble. The toluol solution was concentrated to a small bulk and the material reprecipitated by being poured into a large excess of absolute alcohol. This procedure was repeated five or six times. (A larger number of precipitations did not affect the purity of the compound.) The material was finally dried to constant weight in a vacuum desiccator over calcium chloride.

The results as given in table 1, figured back to cephalin free from calcium, compare very well with the results of previous investigators on the constitution of cephalin, with the exception of those of Levene (36). He obtained a compound possessing a percentage composition, which more closely approximated the suggested formula of cephalin, but he altered the method of purification of crude cephalin.

The ratio of calcium to phosphorus, in our compound, was 1:1, which indicated that one calcium atom was contained in the molecule. The substance was insoluble in alcohol, water, or physiological salt solution but, like cephalin, was soluble in toluol and warm glacial acetic acid.

Coagulative activity. Suspended in physiological salt solution, the calcium lipoid compound possessed no coagulative activity until the calcium was replaced, as by passing carbon dioxide through the suspension or after treatment with dilute hydrochloric acid, which completely removed the calcium, leaving a substance soluble in alcohol and possessing all the chemical and coagulative properties of Bordet's cytozyme as appears in the following experiment:

Experiment 7. Fifty-seven and seven tenths milligrams of sample 2 of the calcium-lipoid compound were dialyzed against normal hydrochloric acid until free from calcium.

	mgm.
Calcium present in sample	2.856
Calcium recovered	2.292

The material was then dialyzed with distilled water until free from hydrochloric acid. The residue in the dialyzing bag was evaporated to dryness and dissolved in about 10 cc. of absolute alcohol. Twenty drops of this solution were evaporated to dryness and taken up in 1 cc. of physiological salt solution. Its coagulative activity was then tested as follows:

Protocol 6. The coagulative activity of the lipoid compound after removal of the calcium with hydrochloric acid

REAGENTS	QUANTITIES		
	cc.	*cc.*	*cc.*
Lipoid compound from sample 2	0.3	0.3	0
Calcified salt solution	0.1	0	0.1
Physiological salt solution	0.3	0.4	0.6
Oxalated guinea pig plasma (0.1 per cent)	0.1	0.1	0.1
Clotting time, water bath, 37°C	6 min. 30 sec.	0	17 min.

These results indicate that calcium chloride reacts directly with the acetone insoluble lipoid extracts in an alkaline medium to produce a cal-

cium-lipoid compound resembling a calcium salt of cephalin. Furthermore, treatment of this compound with dilute hydrochloric acid sets free the calcium and restores the coagulating function to the lipoid.

STUDIES ON THE CHEMICAL STRUCTURE OF THE CALCIUM LIPOID COMPOUND AND THE ACTIVE FREE LIPOID SUBSTANCE. It is evident that the coagulating function of the lipoid is associated with a phosphatid at least similar in constitution and properties to the cephalin of other authors. Furthermore, the calcium salt of this compound contains an amount of calcium which indicates, on the basis of the suggested formula of cephalin, that one atom is present. The fact that the compound easily gives up its calcium on treatment with dilute acid without decomposition of the molecule suggested to us that the calcium probably combines with the phosphate group of the molecule. On account of its strong basic properties, it might replace the organic base, amino ethanol. The possible replacement of organic base by an inorganic base might also account for the difficulty previously experienced in obtaining a pure cephalin. We, therefore, carried out some experiments to determine whether a lipoid substance could be

TABLE 2

Results of the elementary analysis of the calcium-lipoid compound prepared as described in experiment 8

SAMPLE	RESULTS IN MILLIGRAMS PER 100				
	Carbon	Hydrogen	Total nitrogen	Phosphorus	Calcium
3	60.6	9.57	0.6	4.12	5.13

obtained, free from organic base, which possessed the coagulative properties previously ascribed to cephalin.

Preparation of the calcium lipoid compound free from organic base. Experiment 8. First preparation: Dried beef heart tissue was extracted by boiling in a 3:1 mixture of alcohol and ether. The extract was distilled *in vacuo* and the residue taken up in ether. The ethereal solution was poured into a large excess of acetone and the residue again taken up in ether. The concentrated ether solution of these acetone insoluble lipoids was then poured into a very large excess of half normal hydrochloric acid. The precipitated lipoids were collected on soft paper filters and treated with alcohol in which they were completely soluble. This solution was concentrated to a small bulk *in vacuo* and reprecipitated with hydrochloric acid. After resolution of this precipitate in alcohol, it was treated with calcium chloride and ammonia in the same proportion as described for the precipitation of the alcoholic extract of tissue (expt. 6). The precipitate was washed by decantation with concentrations of alcohol increasing from 20 per cent to absolute, then collected on a hard filter paper and, without further purification, was dried at 100°C. in an Abderhalden dryer and analyzed.

As indicated in this experiment, a preliminary treatment of the crude lipoids with hydrochloric acid produced a compound lower in nitrogen than was obtained without acid treatment, which suggested not only a removal of inorganic but also of organic bases from the molecule of the phosphatid. But this preparation was not entirely free from nitrogen.

Experiment 9. Second preparation: A part of the substance prepared in the previous experiment was therefore further treated with hydrochloric acid and redissolved in acetone in which it was almost as easily soluble as in alcohol. This solution, when reprecipitated with calcium chloride and ammonia, and after being washed with water and acetone, was redissolved in toluol, reprecipitated with an excess of acetone, dried in the Abderhalden dryer at 100°C. and analyzed.

The analysis of this compound showed that it was free from nitrogen and corresponded in composition to the assumed formula for cephalin in which the organic base, amino ethyl alcohol, and the available hydrogen of the phosphate group were replaced by a calcium atom. The results of the analysis compared with the theoretical quantities are shown in the table below.

TABLE 3

Results of the elementary analysis of the calcium-lipoid compound free from nitrogen

SAMPLE	RESULTS IN MILLIGRAMS PER 100				
	Carbon	Hydrogen	Nitrogen	Phosphorus	Calcium
4	61.7	9.41	0	2.94*	5.42
Theoretical quantities	62.0	9.68	0	4.22	5.42

* The amount of phosphorus as determined is manifestly low and the result should be confirmed.

Comparative coagulative activity of the different preparations. The coagulative activity of this nitrogen-free product was tested, after removal of calcium by treatment with dilute hydrochloric acid and dialysis, together with the activity of the product obtained in the previous experiment and of the crude acetone insoluble lipoid extracts.

Experiment 10. Portions of the calcium-lipoid compounds 3 and 4 were treated with normal hydrochloric acid, filtered and dialyzed to remove the acid. The material was then evaporated to dryness *in vacuo* and taken up in alcohol.

A saline suspension was prepared from an alcohol solution of the acetone-insoluble lipoids of heart tissue (cytozyme of Bordet). Suspensions of about equal opacity were prepared from the material obtained from samples 3 and 4. The coagulative functions of these suspensions were then tested as shown in protocols 7 and 8.

Protocol 7. Comparison of the coagulative activity of the active lipoid substance from samples 3 and 4 and of the lipoid extract of Bordet (cytozyme) for oxalated rabbit plasma

REAGENTS	QUANTITIES							
	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
Lipoid extract (cytozyme Bordet)	0 3	0	0	0	0 3	0	0	0
Lipoid substance from sample 3	0	0 3	0	0	0	0 3	0	0
Lipoid substance from sample 4	0	0	0 3	0	0	0	0 3	0
Calcified salt solution (0.07 per cent)	0 1	0 1	0 1	0 1	0	0	0	0
Physiological salt solution	0 2	0 2	0 2	0 5	0 3	0 3	0 3	0 6
Oxalated rabbit plasma (0.1 per cent)	0 1	0 1	0 1	0 1	0 1	0 1	0 1	0 1
Clotting time, water bath, 37°C.	12 min.	8 min. 30 sec.	8 min	16 min.	0	0	0	0

Observed for 24 hours.

Protocol 8. Comparison of the accelerating effect of the active lipoid substance from samples 3 and 4 and of the lipoid extract of Bordet (cytozyme) on the coagulation of oxalated rabbit plasma by rabbit serum

REAGENTS	QUANTITIES			
	cc.	cc.	cc.	cc.
Normal rabbit serum	0 1	0 1	0 1	0 1
Lipoid extract (cytozyme Bordet)	0	0 3	0	0
Lipoid substance from sample 3	0	0	0 3	0
Lipoid substance from sample 4	0	0	0	0 3
Calcified salt solution (0.07 per cent)	0	0	0	0
Physiological salt solution	0 5	0 2	0 2	0 2
Water bath, 37°C., six minutes				
Oxalated rabbit plasma (0.1 per cent)	0 1	0 1	0 1	0 1
Clotting time, water bath, 37°C.	12 min.	4 min.	4 min.	4 min.

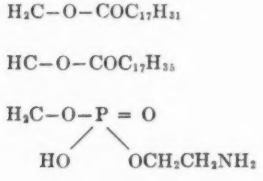
The nitrogen-free product and the others as well, after treatment with hydrochloric acid to remove the calcium, and after dialysis, possessed coagulative properties quite similar to those of the lipoid extract, cytozyme of Bordet; namely, the acetone-insoluble lipoid extracts from which they were derived. Thus it would seem, from these results, that the coagulative activity of this compound, with the structure indicated in formula 3 below, is closely associated with the acid properties of the phosphate group of the lipoid molecule.

Chemical structure of the active lipoid substance. Since the preparations free from nitrogen possess the coagulative function of the crude products from which they were derived, it may be suggested that the amino ethyl

alcohol radical and the available hydrogen atom of the phosphate group of cephalin (formula 1) might both be replaced by the calcium atom (formula 2) and that the active lipoid essential to the process of coagulation might have the constitution represented by formula 3.

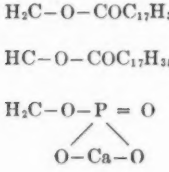
Formula 1

(Cephalin)



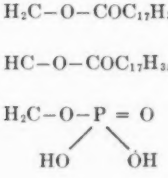
Formula 2

(Replacement by calcium)



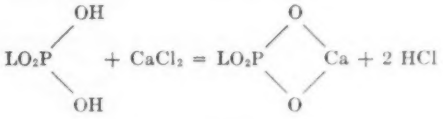
Formula 3

(Replacement of calcium by action of acid)



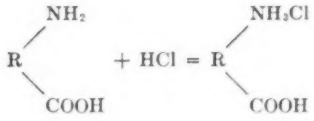
It also seems evident that ionized calcium, that is calcium chloride, reacts chemically with a lipoidic substance to produce a definite calcium-lipoid compound. This active lipoidic substance possesses available hy-

drogen which is apparently replaced by the calcium of the calcium salt to form acid (HCl) as indicated in the following equation:



Thus, it is suggested that in the coagulation of the blood, ionized calcium salts react with the active lipoid substance to form a relatively insoluble calcium-lipoid complex and liberate acid, which, in turn, is neutralized by available substances in the plasma, among them protein.

STUDIES ON THE REACTION BETWEEN THE ACTIVE LIPOID SUBSTANCE AND THE PROTEINS OF PLASMA AND SERUM. According to Loeb (37), protein reacts stoichiometrically with acid to form protein salt, as indicated in the following equation:



When varying quantities of dilute hydrochloric acid are added to plasma, a point is reached at which a flocculent precipitate is formed; also, slight acidification of dilute plasma with hydrochloric acid or carbon dioxide gas results in the formation of a precipitate. This precipitate, when redissolved in salt solution and calcified, coagulates to form fibrin. If serum is diluted and acidified in a similar manner, a precipitate of euglobulin results. It occurred to us that these precipitates might represent complexes of protein with the active lipoid substance which developed as a result of the acidification of the plasma or serum. Accordingly, the effect of adding lipoid to plasma or serum in the presence of dilute hydrochloric acid was studied in the following experiment:

Experiment 11. Horse serum and 0.1 per cent oxalated sheep plasma were diluted 1:20 with distilled water. A saturated solution of lipoid substance was prepared by concentrating an alcoholic solution of the acetone-insoluble lipoids prepared from beef heart tissue. An excess of the lipoid substance was found to precipitate the proteins of plasma and serum completely from solution and such mixtures were found to be acid, due to a natural acidity of the lipoid. When a quantity of lipoid was added, insufficient to neutralize the alkalinity of the plasma or serum, no precipitate was formed. In the presence of a trace of an added acid (HCl), however, any amount of lipoid would produce a precipitate of the plasma or serum. It was found, in fact, that if the mixture was kept very slightly acid, definite proportions of lipoid and protein were mutually and completely precipitated from solution as a

lipoid-protein complex. Such a mutual precipitation of the proteins of plasma and serum with lipoid, together with the necessary controls, is shown in the following protocol:

Protocol 9. Mutual precipitation of the proteins of plasma and serum with active lipoid substance after acidification

TUBE NUMBER	LIPOID SOLUTION	ABSOLUTE ALCOHOL	N/10 HCl	N/10 NaOH	DISTILLED H_2O	PLASMA	SERUM	RESULT
1	2.0	0	0.05	0	8.95	5.0	0	Heavy precipitate
2	2.0	0	0	0.5	8.50	5.0	0	No precipitate
3	0	2.0	0.05	0	8.95	5.0	0	Slight precipitate
4	0	2.0	0	0.5	8.50	5.0	0	No precipitate
5	0	0	0.05	0	10.95	5.0	0	Slight precipitate
6	0	0	0	0.5	10.50	5.0	0	No precipitate
7	0	0	0	0	11.00	5.0	0	No precipitate
8	5.0	0	0.1	0	5.9	0	5.0	Heavy precipitate
9	5.0	0	0	1.0	5.0	0	5.0	No precipitate
10	0	5.0	0.1	0	5.9	0	5.0	Slight precipitate
11	0	5.0	0	1.0	5.0	0	5.0	No precipitate
12	0	0	0.1	0	10.90	0	5.0	Slight precipitate
13	0	0	0	1.0	10.00	0	5.0	No precipitate
14	0	0	0	0	11.00	0	5.0	No precipitate

Heavy precipitates were formed in tubes 1 and 8 and slight precipitates in tubes 3, 5, 10 and 12. No precipitates were formed in the other tubes. The precipitates were filtered off and the solutions tested for their compara-

TABLE 4

Results of the analyses of the protein and the phosphorus in the solutions of the previous experiment after removal of the precipitates

TUBE NUMBER	PROTEIN	PHOSPHORUS	TUBE NUMBER	PROTEIN	PHOSPHORUS
	gr./liter	*mgm.*		*gr./liter*	*mgm.*
1	None	0.05	8	None	None
2	2.70	10.82	9	3.90	27.05
3	2.16		10	1.80	
4	2.70		11	3.90	
5	2.16		12	2.50	
6	2.70		13	3.90	
7	2.70		14	3.90	

The picric acid reagent of Esbach, and Millon's reagent gave negative results in the tests for protein in the solutions in tubes 1 and 8.

tive protein content by the nephelometric method of Kober (38), using sulphosalicylic acid as the precipitant. The total protein present in the

dilutions of plasma and serum was estimated from total nitrogen determinations. Tubes 1 and 8 were duplicated so that the lipoid could be estimated by comparative determinations of phosphorus in the amount of lipoid solution used and in these solutions after precipitation. The results are indicated in table 4.

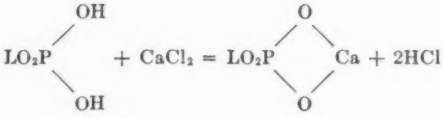
These results demonstrate quite clearly that the proteins of plasma and serum react with the lipoids to form complexes which precipitate from aqueous solution when properly acidified. It is evident that the acid so alters the protein as to make it susceptible to the reaction with the lipoid. It is also evident that, when certain definite quantities of protein and lipoid were used in the experiment, the precipitation was mutual and complete—no trace of either protein or lipoid remaining in solution. Protein was also completely precipitated by an excess of lipoid without acidification other than that due to the natural acidity of the phosphatid. When only small quantities of lipoid were added to plasma or serum, without acidification, or when a mixture containing an excess of lipoid was kept neutral by the addition of a little alkali, no precipitation occurred. Pseudoglobulin and albumin fractions of serum were also completely precipitated from solution by similar treatment. Thus, it is evident that not only fibrinogen but all the proteins of plasma, after proper acidification, react with the lipoid to produce lipoid-protein complexes which precipitate from solution.

THE MECHANISM OF THE CHEMICAL REACTIONS UNDERLYING THE COAGULATIVE PROCESS. In this experimental study, certain definite chemical reactions taking place between the factors essential to the coagulation of the blood, have been demonstrated. Ionized calcium salts react with active lipoid substance to produce a calcium-lipoid complex and acid, and the proteins of plasma react with lipoid in the presence of acid to produce an insoluble lipoid-protein complex. Since, in the coagulation of the blood, the amount of calcium required is very small, and the amount of acid it would therefore be able to produce very minute, and, further, since ionized calcium is found in almost undiminished amount in the serum after formation of the clot, the calcium salt, in all probability, functions as a catalyzer, taking part in the reaction but being subsequently reformed to appear in the serum. Moreover, contact is an important factor. In contact catalysis, the surface of contact, exclusive of any specific part it may play as a result of its chemical nature, may accelerate a reaction by concentrating upon itself by adsorption the chemically reactive substances which may be present in the solution. It is therefore suggested that this adsorptive function of the surface probably accelerates the reactions concerned in the formation of fibrin. These chemical reactions may be divided into three steps as follows:

When the active lipoid substance meets the ionized calcium salt, cal-

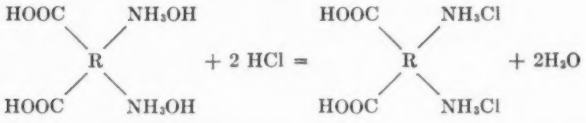
cium chloride, at a surface of contact, the reaction theoretically may be supposed to occur as illustrated in equation 1.

Equation 1



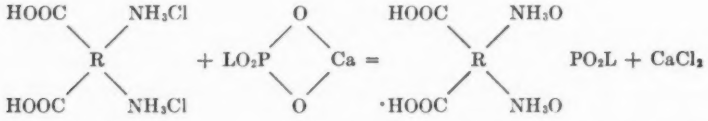
If the acid set free as a result of this double chemical decomposition were neutralized, the calcium salt of the lipoid would be formed. In the presence of acid, this calcium lipoid compound retains its soluble and probably ionized state. The same surface of contact has also concentrated upon it some of the fibrinogen with which the blood or plasma is saturated. As soon as some free acid is formed, as the result of the reaction pictured in equation 1, it reacts with the fibrinogen to produce acid fibrinogen as shown in equation 2.

Equation 2



Fibrinogen reacts with lipoid in an acid medium to produce an insoluble lipoid-protein complex. If the calcium-lipoid complex, indicated in equation 1, is in the soluble or ionized state, which it must be in the presence of acid, the reaction which brings about the formation of the insoluble lipoid-protein complex may take place as illustrated in equation 3.

Equation 3



Thus, in the equation, the ionized acid protein reacts with the ionized calcium lipoid to produce an insoluble lipoid-protein complex in the formation of a film of fibrin, and liberates again the ionized calcium salt which becomes available for further reaction with active lipoid substance.

It is not to be inferred that the reaction necessarily takes place in the exact manner illustrated in these equations. They are suggested as a not improbable explanation of how the reaction would occur whenever the reacting substances become concentrated at a surface of contact. The

lipoid alone is not sufficient to induce the catalytic reaction even in the presence of calcium and fibrinogen. It requires the accelerating influence of another factor—contact. When, however, all these substances are present, and calcium reacts with lipoid to produce acid on a surface in contact with fibrinogen, ionized complexes are formed of both lipoid and fibrinogen together with the ionized calcium salt and acid. The actual formation of the lipoid-protein complex—fibrin—depends upon the greater affinity of the lipoid ion for the protein ion and the relative insolubility of this complex under the existing conditions. In other words, all the reactions probably do not come to completion in the three distinct stages, but rather the reaction of calcium with lipoid and the resulting formation of acid gives rise to the formation of active ions which, when concentrated by adsorption on the surface of contact, react to form the lipoid-protein complex until equilibrium is reached in the plasma. The residue of these ions together with a trace of the unprecipitated lipoid-protein complex, may conceivably possess the coagulative function attributed to the so-called "ferment thrombin" of previous observers.

Fibrinogenesis thus appears to be a very striking illustration of a catalytic process, hitherto attributed to an enzyme, in which it is now possible to recognize certain definite chemical reactions. This catalytic reaction may not only afford a true explanation of the mechanism of the coagulation of the blood but it may also form the basis for the investigation of many biological phenomena which are at present obscure, and especially for an approach to a study of the relationship of various proteins of the blood to the lipoids within the cells in the adaptative processes of nutrition and immunity.

In view of the fact that only a thin cellular membrane separates the protein, foreign or native, and other substances circulating in the blood, from the lipoid of the cells, and this membrane varies in permeability under varying conditions of cellular activity, the catalytic process above described suggests a mechanism of exchange between the tissues and the blood which opens up a new field of study. Moreover, this form of chemical catalysis may possibly underlie other processes seemingly fermentative, or even enzyme action in general.

SUMMARY AND CONCLUSIONS

Previous work has shown that three definite substances, that is, fibrinogen, ionized calcium salts, and a lipoid (cephalin) are concerned in the coagulation of the blood and that contact is a determining factor.

Various hypotheses have been developed to explain the mechanism of the process. Of these, the most generally accepted attribute the coagulation of the blood to the transformation of fibrinogen into fibrin by means of

a ferment—thrombin. In the blood, the thrombin is supposed to exist in an inactive form, being converted into the active ferment when blood is let due to the action of contact, calcium ions and a lipoid (cephalin).

In this study, the solutions of proferment, serozyme of Bordet, which he supposed to be free from fibrinogen, were found to contain fibrinogen. The stability of phosphate plasma, which failed to coagulate in the presence of lipoid extracts and calcium ions, and which he attributed to the removal of (serozyme) proferment was found to be due to the presence of free alkali and alkali phosphates resulting, presumably, from the decomposition of tricalcium phosphate.

In this study of the coagulation of the blood, two essential reactions were recognized: one, in which ionized calcium salts react with an active lipoid substance to form a calcium lipoid compound and acid; the other, in which not only fibrinogen but all the proteins of the blood as well, when slightly acidified, apparently combine in definite proportions with the active lipoid constituent to form a lipoid-protein complex which precipitates, leaving the solution entirely free from both protein and lipoid.

A lipoid substance of tissue which possessed active coagulative properties was isolated from the acetone insoluble extracts of tissue by precipitation of its calcium salt. The elementary analysis of this calcium salt corresponded closely with the percentage composition of cephalin in which the organic base, amino ethanol, was replaced by one hydrogen atom.

Based upon this study, a process of chemical catalysis in which the ionized calcium salts function as the catalyzer under the accelerating influence of contact, was described, which not only may explain the mechanism of coagulation, but suggests an approach to the study of the relationship of various proteins of the blood to the lipoids within the cells in the adaptative processes of nutrition and immunity and possibly also of cellular pathology.

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THE RÔLE PLAYED BY THE SIZES OF THE CONSTITUENT FIBERS OF A NERVE TRUNK IN DETERMINING THE FORM OF ITS ACTION POTENTIAL WAVE

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Numerous experiments, performed in this laboratory during the last few years, upon the form of the action potential wave of nerve, have established the fact that the wave is built up of units, which we have designated as axon potential waves. The form of the potential-time curve, as led off from a point on the nerve, depends in part upon the distance of this point from the point stimulated, and in part upon the composition of the nerve. Some nerves, as for instance the phrenic or motor spinal roots, always give simple waves (Erlanger, Gasser and Bishop, 1924, 1926); others, as for example the sciatic or the sensory spinal roots, show definite secondary waves which for convenience we have called α, β, γ and δ. The particular action potential pattern which is characteristic for each nerve, depends upon the velocities of the constituent axon action potentials involved.

When many fibers of low velocity are present, as occurs in the posterior roots, the action potential becomes much elongated on conduction; in the absence of such fibers, as is the case in the anterior roots, the action potential is much shorter.

In the nerves and roots whose action potential curves show secondary waves, these waves are not themselves definite entities, but also change their form as they are propagated. They are made up of axon action potentials of varying velocities; in fact the slowest fibers of the α group are about as slow as the fastest fibers of the β group, and the slowest fibers of the β group show the same relationship to the fastest fibers of the γ group. The nerve thus seems to be composed of fibers whose velocities of conduction form practically a continuous series from the fastest to the slowest. The continuity of the series is indicated by the growth of the area of the recorded wave as the stimulus is increased in size. The growth is continuous, but at varying rates, as the stimulus is increased, taking place either without gaps or with gaps corresponding to a very short range of stimuli (1926). The appearance of secondary waves cannot therefore

be produced merely by the presence of fibers of widely different velocities of conduction but there must be variations in the numbers of the fibers at each velocity.

The purpose of this paper is to investigate the morphology of the different nerves, to see if any relationship can be ascertained between their structure and their action potential curves.

Before proceeding further with this question we must point out collateral lines of evidence focusing upon the solution of the problem. Some of these were reviewed in our previous communication (1924) in the discussion of the possible explanations of the wave form. It was stated by us at that time that "it is not at all surprising, in view of the varied histological composition of mixed nerve, that the action current of such a nerve as the sciatic is compound."

In 1913 Lapicque and Legendre established experimentally the relation between the chronaxie of nerve and its morphology. While they considered the possibility of chemical or colloidal factors as variables in the nerve's composition, they felt that the first series of observations should be directed along simpler lines, and they compared the time constants of different nerves with their gross histological aspects. The research led very definitely to the conclusion that the factor relating chronaxie to nerve structure was the size of fiber, and that this operated in such a way that the larger the fiber the shorter the chronaxie. Knowing that the chronaxie has a relation to the velocity of conduction they say, ". . . . les fibres nerveuses sont d'autant plus rapides qu'elles sont plus grosses." The exact function of the size of fiber was not determined, though it was suggested that it might be the area. More recently Langley as the result of some histological studies on nerve constitution, expressed the opinion that one of the main factors accounting for the extraordinary variations in the size of the myelinated fibers is the nature of the tissue in which they end. This opinion, taken with the older observation by Carlson that the velocity of propagation in a motor nerve depends upon the speed of the muscle innervated, again brings an association between velocity and size. Also, from a theoretical consideration of how a cable with the dimensions and constants of nerve would conduct, Göthlin has pointed out that a large strand would conduct faster than a small one. Such a calculation does not, however, establish any relationship between nerve size and velocity unless a cable like conduction can be demonstrated. As will be seen later, there is no evidence that such a relation obtains.

When Professor Lapicque was shown the cathode ray oscillograph records of the action potential wave in nerve, during a visit of one of us to the Sorbonne, he expressed an opinion very strongly in favor of the possibility that the form of the wave would depend upon the sizes of the fibers in the nerve. The two most striking cases known at that time in mammalian

nerve were the phrenic wave, which is simple, and the saphenous, which is compound. On investigating these nerves histologically (Lapicque, Gasser and Desoille) it was found that their microscopic appearances were as notably different as their electrical signs. The phrenic nerve presented a relatively homogeneous appearance; the saphenous was composed of fibers of widely varying sizes.

Such an observation was very encouraging and a further effort was made to find more exactly the relationship between the distribution of fibers and the action potential wave. After various trials the function of the size which seemed the most probable in determining their propagation velocity was found to be the diameter of the fibers. At this point the researches at the Sorbonne were interrupted, and after a year the problem was again taken up in the Washington University laboratory where it was possible to make electrical observations upon the same nerves that were to be examined histologically.

The analyses have necessitated three procedures: the measurement of the nerve fibers, the recording of their action potential waves and the theoretical reconstructions of the potential wave forms, on the basis of the fiber distribution in the nerves, for comparison with the actual waves as recorded.

The measurement of the nerve fibers. After the action wave was recorded the nerve was removed from the moist chamber, tied to a piece of glass so as to maintain as nearly as possible its natural length, then fixed in 1 per cent osmic acid. Paraffin sections 4μ thick were prepared,[1] care being taken that the section was at right angles to the nerve. These were photographed, and the negatives then enlarged so that finally a print was obtained at 1000 or more diameters. During the process of experimentation and fixation some distortion of the fibers took place so that they had a tendency to appear ellipsoidal. On account of the nature of the problem it was impossible to prepare large numbers of nerves and select the best ones; it was necessary to make the best utilization possible of those nerves whose potential waves had been recorded. The routine procedure was, therefore, to make two measurements of each fiber, one through the long axis and the other through the diameter at right angles to this. The mean diameter of the fiber was then obtained by extracting the square root of the product of the measurements. The product itself was a figure representative of the cross-sectional area. The outside diameter only was measured in the case of most nerves, because it was shown by Lapicque and Legendre (1922) that in injured nerves the myelin swells at the expense of the axis cylinder. If, as Donaldson and Hoke

[1] We are indebted to Professor Ranson for permission to have the histological sections prepared in his laboratory.

claim, the ratio of the thickness of the myelin to the fiber diameter be a constant for all sizes of fibers, then for comparative purposes the outside diameters are as good as the inside diameters with the advantage that there is less danger of pathological distortion. We have not made a rigid investigation of Donaldson and Hoke's observation, but measurements of our material do show some deviation from the constancy of this ratio. Such a deviation in so far as it occurs is a source of error, but where it occurs in very small fibers, it is of little importance as it will be seen that the small fibers contribute very little to the total area of the potential-time curve obtained from the nerve. In the small number of cases where both the total diameters and the diameters of the axis cylinders were measured the resulting size-distribution curves showed no significant deviation one from the other. In some nerves all the fibers were measured, in others only a number such that further additions would be of no important statistical significance.

Due to distortion of fibers or proximity to a node of Ranvier a single measurement of a fiber might deviate somewhat from the mean diameter. To gain some idea of this error a number of fibers were followed through five sections. As the result of this it was found that the probable error of a single result, as determined by comparison with the mean of the five measurements, varied between 2 and 8 per cent. This error would not be sufficient to interfere with any conclusion that is drawn from the investigation.

In order that an analysis of a nerve on the basis of fiber size can be made at all there must be sufficient constancy of diameter throughout its course. Sherrington quotes Schwalbe to the effect that the diameters of the motor nerve fibers remain unaltered throughout their course except when branching occurs, and branching does not occur, according to Mays, either in the nerve trunks or the sciatic plexus of a frog, a fact confirmed by Sherrington. Schwalbe on the other hand states that the sensory fibers across the dorsal lymph sacs of frogs decrease in size in their course. If tapering occurred in our nerves to any considerable extent, then the number of large fibers in the peripheral nerves should be smaller than one would expect from the large fiber content of the spinal roots, and this is not obviously the case as can be seen by comparison of figures 2, 4, 8 and 9 of spinal roots, with figures 6 and 10 of peripheral nerve; so here again we may infer that tapering of the fibers within the nerve trunk is not of a disturbing magnitude.

The unit action potential wave. In the reconstruction of nerve trunk potentials it is necessary to define the dimensions of the unit axon potential both as to magnitude and duration.

The *magnitude* of the unit wave was selected as constant for all sizes of fibers. This arbitrary assumption was necessitated by the complete absence of any data indicating its value, and whatever justification it may

have lies in the *a posteriori* reflection that a group of fibers, which in the analyses seems to produce a secondary wave, would not produce one of the proper magnitude if the individual potentials were assigned any other value.

Although we have assumed the potentials developed in the different fibers to be the same, this does not mean that they will have equal effects upon the recording mechanism, in fact there is reason to believe that their values would correspond more closely to their cross-sectional areas, which determine the electrical resistances per unit length.

The potential drops across a recording instrument produced by two sources of potential having the same E.M.F. but different resistances will be to each other inversely as the resistances, only when the resistance of the recording instrument is low compared with that of the source. A vacuum tube properly adjusted releases power according to the potential of the grid; and the resistance in the grid circuit is very large so that the tube acts as a potentiometer with considerable independence of the resistance of the source. But, on the other hand, the resistance of the axon of a single nerve fiber must also be a very large quantity. Its value can only be calculated; but if one take as the specific value of the resistance of the axon protoplasm that of blood serum, in round numbers 98 ohms (Bugarsky and Tangl), the resistance of a nerve fiber turns out to be between 10^8 and 10^9 ohms per centimeter depending upon the size of the axon. These figures accord very well with the value, given by Göthlin, of 10^9 ohms for a small fiber, using the specific resistance of cerebrospinal fluid which he determined for the purpose. If we assign to the grid circuit a value of 2,000,000$^\omega$, then the resistance of a single fiber is so much higher, that the potential drop across 2,000,000$^\omega$ would be determined mainly by the nerve's resistance.

As will be seen in the actual analyses, there is reason to believe that it is possible to reveal in the action potential wave the effect of only a few large fibers, so that a consideration of the effect of a single fiber on the potentiometer is of some practical interest. In addition to the resistances considered above there is a third factor of considerable importance in determining the effect of a fiber. An active fiber or a group of active fibers of the same size is shunted by the inactive fibers and the other structures in the nerve. We may picture the situation as is indicated in the accompanying diagram (fig. 1). The active fiber developing a constant potential, E, but having a resistance, r, depending upon its cross section, is shunted by the remaining parts of the nerve, R_1, and is recorded on a potentiometer with a resistance R_2. The potential drop across R_2 will then be

$$\frac{R_1 R_2 E}{r(R_1 + R_2) + R_1 R_2}$$

This expression is symmetrical with respect to R_1 and R_2; therefore the effect of the inactive portions of the nerve is the same as that of a low resistance potentiometer in making the recorded E.M.F. a function of the resistance of the source. If one assume 100 fibers each of the 10^9 and the 10^8 ohm sizes to be active in a nerve whose total resistance is around 50,000$^\omega$ per centimeter, and that their potentials are recorded on a potentiometer having 2,000,000$^\omega$ internal resistance, using leading off electrodes two centimeters apart, then the ratio of the potentials recorded will only vary 4 per cent from the ratio of the resistances. In view of these considerations it was routinely assumed in making reconstructions that the potentials recorded were a linear function of the areas of the fiber cross-sections.

The duration of the unit wave. For the sake of convenience the shape of the axon potential curve has been assumed to be triangular and the duration the same for all fibers of the nerve trunk. The potential curve may be simplified to a triangle of nearly equivalent area, which can be much more easily manipulated in the analyses. The durations were chosen as the same in all fibers because they seem to be constant in actual measurements made at the stimulating cathode (1926).

The values for the duration of the rising phase of the potential wave and for the total duration are taken from actual data. When leads are made from the stimulating cathode on the phrenic nerve of the dog at 37° C., rising phases of about 0.3 σ and less are found, which include whatever utilization periods there may exist, and total durations of about 1.0 σ, measured to the point where the potential has practically returned to the base line (1926). The start of the action potential even at the stimulating cathode has an initial upward concavity, and similarly and to a very much more marked extent the potential dies away asymptotically. Triangles drawn with the crest, the start and the end of the action wave, as apices, would include an area much larger than the actual wave, a deviation which would be theoretically undesirable; on the other hand triangles drawn with their legs having the principal slopes of the rising and falling phases would have areas differing but slightly from the actual ones. Accordingly the latter procedure was adopted and when this was done, 0.2 σ seemed a good value to take for the rising phase and 0.6 σ for the total duration. For cold blooded nerve similar considerations led to the adoption of 0.3 σ and 0.9 σ as the proper values.

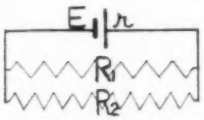


Fig. 1

The velocities of the unit waves in terms of fiber diameters. In searching for the connection between these two values the cable-like construction of the nerve was taken into consideration. The existence of protoplasmic

strands surrounded by a sheath having some insulating value has from time to time led physiologists to consider nerve conduction in the light of the physics of submarine cables. While it has been calculated that in a cable of strands having the dimensions of nerve fibers, the velocity of conduction would be of the same order of magnitude as in a nerve fiber, provided the length be suitably chosen, the analogy seems to end here. The conduction time in nerve is a linear function of the length and not a function of the square of the length as in a cable; contrary to what takes place in a cable, the size of the wave in an axon is independent of the shape and size of the impressed potential; decremental conduction present in a cable is absent in nerve (Kato; Davis, Forbes, Brunswick and Hopkins); and, whereas high frequency unidirectional currents applied to a cable cause it to deliver a constant current, the nerve responds repetitively according to its intrinsic properties (Eigenperiode).

Most theories of nerve conduction postulate that some of the energy liberated in an active portion of a nerve is responsible for the activation of the adjacent inactive portion, the process thus being carried on without decrement due to the energy added to the disturbance at each successive point in its progress. The particular form of this type of theory which has the most adherents is the one which considers that the action current is the agent of excitation, especially as this theory has been elaborated by Lillie. It might be argued that while propagation in nerve is quite different from what occurs in a cable, that in so far as propagation is brought about by the action current it could be modified by the nerve's cable-like structure. This seems to be essentially the attitude taken by Göthlin (1907).

According to the telegraph equation of Lord Kelvin, the time of arrival of the wave front at the end of a cable, after the closure of a key at the sending station, is CRl^2 times a constant, where C and R denote respectively the total capacity and resistance of the circuit, and l is its length (Fleming, p. 154). The capacity per unit length of a cylindrical conductor of radius R_1, placed concentrically in the interior of a conducting cylinder of inner radius R_2 and separated from it by a substance having a dielectric constant K, is $\frac{K}{2\ ln\frac{R_2}{R_1}}$. According to Donaldson and Hoke, the ratio of the myelin to the total diameter of the fiber is constant. In so far as this is true, the ratio $\frac{R_2}{R_1}$ would be constant for all fibers and their capacities would be constant except for variations in the composition in the myelin. In any case, then, the capacity factor could alter the propagation rate only as the myelin sheaths might be of different qualities. Actually it will be seen that the action potential waves can be satisfactorily reconstructed

without supposing that the myelin sheath enters in as a controlling factor at all.

The other factor determining rate of propagation in a cable is the resistance, that is, the smaller the resistance the faster the conduction. The operation of this factor in the case of nerve would lead one to infer that the velocities of conduction would vary as the cross-sectional areas of the fibers, but reconstructions made on this basis yielded results differing widely from the facts. On the other hand velocities varying among one another as the diameters of the fibers were found empirically to lead to the synthesis of action potential waves very much like those recorded. This relationship was therefore used in all the reconstructions in the following section. As far as cable conduction is concerned, its consideration yielded no clews useful in the theoretical analysis of the potential curves.

THE ANALYSES. These involved the measurement and tabulation of 10,500 fibers in various nerves.

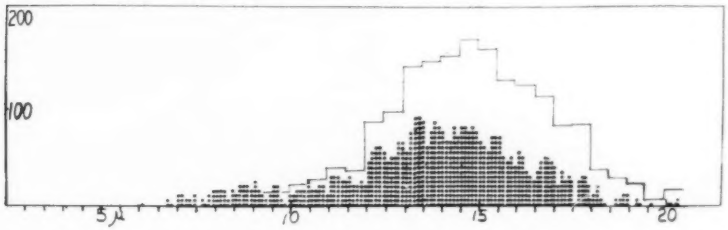


Fig. 2. Distribution curve of all the fibers (875) in the IX*th* motor root of a bullfrog. Each dot is a fiber. Solid lines: total area of the underlying fibers. The ordinate in this and similar figures is on an arbitrary scale which is not the same in the different figures.

The first group of nerves which will be reported consists of the IX*th* pair of roots of a bullfrog (*Rana catesbiana*) together with the peroneal from the same animal. The roots were studied after the peroneal nerve, and the frog had been in the refrigerator at 2°C. during the interval of two hours. This may explain the velocity of conduction which was found to be 35 m.p.s., somewhat less than usual. When the nerve and roots had been dissected they were mounted in the moist chamber at room temperature. A stimulating electrode was placed 48 mm. from the central end, this being the longest distance usable before important branching took place. Stimulation of practically all the fibers was thus assured. A long series of records was taken from each root in turn at graded strengths of stimulation, after which the roots were fixed for histological examination.

The motor root. As is well known, the motor root has a rather homogeneous histological appearance compared with the sensory. This is

due to the large mass of fibers which centers about 14 μ, as seen in figure 2. In this figure and in others of the same kind each dot represents a nerve fiber. It is located along the abscissa at its proper mean diameter and by counting in the direction of the ordinate, the number of the fibers at the size may be determined; the figures therefore serve as a form of tabulation. The solid lines in steps represent the total areas of successive groups made up of fibers within a range of 0.5 μ. In this motor root all the fibers (893) were measured except the very small ones beyond the left end of the series,

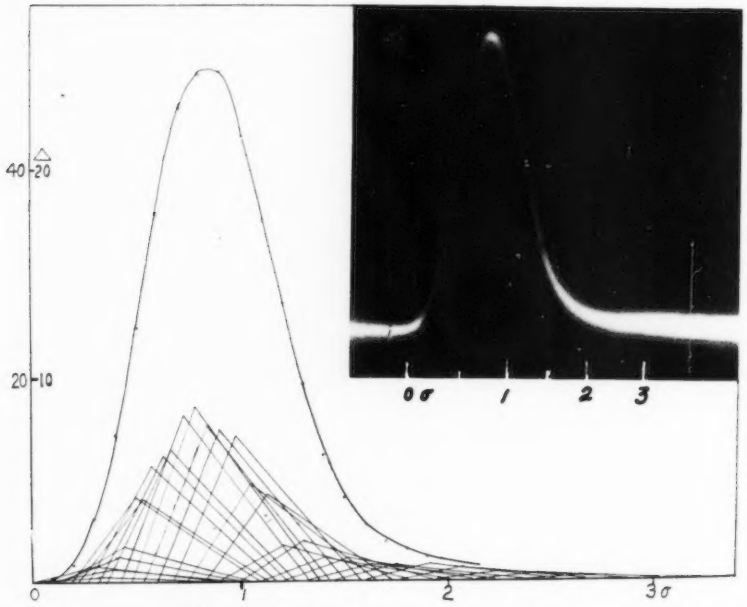


Fig. 3. Reconstruction of motor root action potential at 48 mm. of conduction. The triangles are plotted on twice the scale of the summation line.

Inset: action potential of the motor root as recorded (10/27/25). 1 mf., 4020$^{\omega}$, X = 87 mm.

which are presumably the preganglionic fibers to the sympathetic system. The striking point about this chart is a single peak at 14 μ, with the number of fibers decreasing to either side. The largest fibers are just over 20 μ in diameter. These were considered to have the maximum velocity of 35 m.p.s. The other velocities were calculated according to the diameters of the groups, and then the lag of each group was determined when the 20.25 μ group had reached a point 48 mm. from the start, which was the distance at which the records were taken.

In figure 3, the action potential corresponding to each group was plotted at its proper delay, marked out along the abscissa, with triangles whose height was determined by the total area of the group. The sum of these then made the reconstructed composite action potential, for comparison with its real counterpart. As would be expected from the regular fiber distribution, the curve is perfectly smooth. The real action potential is inserted for comparison. The correspondence is quite satisfactory. The crest time of 0.84 σ in the reconstruction compares well with 0.76 σ measured on the record; also the endings are in accord, the potential having returned nearly to zero in both at 3 σ.

The sensory root. When we turn now to the sensory root (fig. 4), all of whose fibers were measured, we find a very different picture. The range of sizes is about the same, the largest being just over 20 μ, but there is a relatively much smaller number of fibers above 15 μ than in the motor

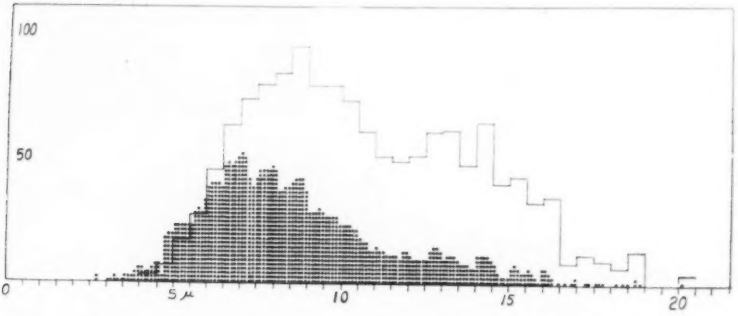


Fig. 4. Distribution curve of all the fibers (1577) in the IX*th* sensory root of the bullfrog.

root. The peak in the number comes at 7 μ, but on the basis of area the maximum comes at 8.5 μ. Furthermore, a mere glance at the figure reveals that the area of the fibers smaller than 11.5 μ is greater than that of those larger. The effect of this appears quite clearly in the action potential which is recorded in figure 5. Upon the reconstructed action potential is redrawn in linear coördinates a maximal action potential from the root. It can be seen that the smaller fibers are definitely responsible for the β wave. Attention should be drawn to the fact that in figure 4 the peak of the area of the smaller fibers is higher than the peak of the larger fibers, while in the reconstruction and in the actual action potential, the β wave is lower than the α. The reason for this is that according to the hypothesis the axon potentials of the smaller fibers separate out more rapidly than those of the larger ones, therefore fewer of them are summed at any one time.

One source of error occurs in the posterior root which is absent in the anterior, that is, the delay which has been found in the ganglion, amounting to an average of 0.14 σ (1926). Whether or not it is the same for all fibers is not known. In any case no correction could be made for it. But in spite of the fact that the conduction time in the fibers of the same size in the two roots has been taken to be the same, the agreement between the real action potential and the reconstruction is sufficiently satisfactory both as to shape and time constants. The differences are best described by the figure itself.

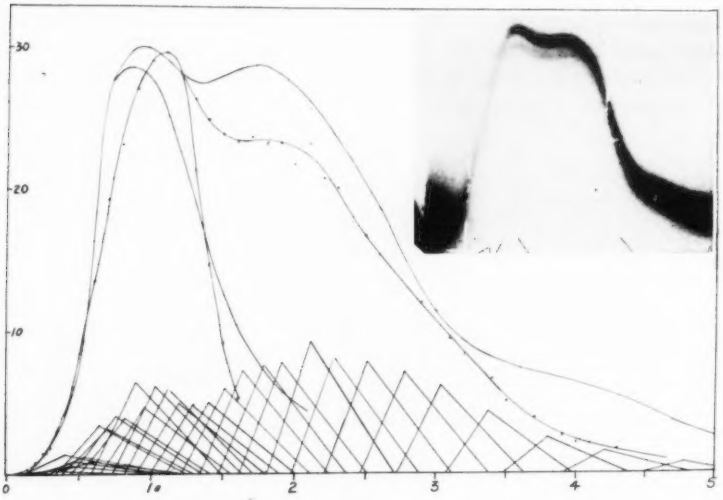


Fig. 5. Reconstruction of action potentials in posterior root of bullfrog at 48 mm. of conduction. ○○○○, reconstruction; long curve, whole nerve; short curve, fibers above 11.5μ. ————, action potentials from the nerve redrawn in linear coördinates; longer curve, maximal; shorter curve, submaximal.

Inset: maximal action potential of nerve as recorded (10/27/25). × 0.7, 1 mf., 4020$^{\omega}$, X = 87 mm.

Starting with the larger fibers, the area of the first 15 groups in figure 4, as subtended by the rectangles, is 38.8 per cent of the area of all the fibers. The reconstructed action potential of these fibers alone has been added to figure 5. From the series of action potentials made from this nerve, the strengths of stimuli were plotted against the areas of the curves, the areas being measured with the aid of a planimeter on large scale redrawings of the originals, in rectangular linear coördinates. The area curve showed an acceleration at the point at which the β fibers began to respond. It happened that the last potential curve before this acceleration had an area

38.3 per cent of the maximum; accordingly, it has also been inserted in figure 5 to show its agreement with its reconstructed counterpart. In the introduction the reason for considering that a fiber affects the galvanometer as its area was presented. The agreement between theory and fact in this experiment may be taken as experimental evidence of the correctness of the assumption. It also shows that the fibers larger than 11.5 μ are responsible for the α wave and that the area of the β group also corresponds with the fibers belonging to the second peak in the analysis (fig. 4).

The peroneal nerve. The fibers in the preparation of this nerve turned out to be quite wrinkled, thus making the measurements less accurate. However, there appear definitely three groups (fig. 6), the first two corresponding to the α and β groups which were analysed in the roots and the third corresponding presumably to the γ fibers. As this nerve had the fastest velocity of conduction of the three (43.2 m.p.s.), because it was studied under the most favorable conditions, its largest fiber was credited with this velocity instead of the value taken for a fiber of the same size in the roots. The forms of the action potential and of the reconstruction have a superficial resemblance. The deviation (fig. 7) consists in an earlier appearance of every point on the reconstruction as compared with the corresponding real point. This means that the small fibers are conducting relatively more slowly than that calculated on a diameter basis. The peroneal is a fine nerve and it is possible that it received some injury in dissection. While we know very little about the relative susceptibilities of fibers of different sizes to injury, and while we have had little experience with peroneal nerves, we do know that the spread of fibers as indicated by the crest time is greater than appears in the sciatic data published in 1924. The potential wave was recorded at 146 mm. of conduction; but at 80 mm. of conduction the crest time was 1.1 σ, which may be compared with 0.8 σ, an average of the published values in the sciatic at that distance. The supposition that tapering occurs in the fibers would not aid in the explanation and the sizes recorded in figure 6 are not in favor of its existence.

In figure 7, there is an instance of the dependence of the wave form on the distribution curve. There is an extra hump on the rising phase and this also appears in the recorded potential.

In the distribution curve every size is represented. When the action potential was built up in 18 successive steps by increasing the strength of stimulation, no point could be found in which additional fibers could not be added to the total. While injury of fibers might tend to fill a gap in the electrical picture, and the error in measuring diameters one in the distribution curve, the results clearly indicate that there is no marked free interval in either. Also addition of the fibers in the two roots does not

indicate that there would be free spaces in the distribution curves of a mixed nerve.

In order to prove that the analysis of the IX*th* pair of roots may be taken as typical of roots in the bullfrog, partial analyses were made of the seventh and eighth pairs of another frog. These analyses, which are put on record in figures 8 and 9, indicate that essentially the same conditions obtain in the other roots. In a previous paper on the spinal roots (1926) it was stated that the velocities of conduction in the motor and sensory roots are essentially alike and that if there be any difference, the sensory root contains fibers of lower threshold and higher velocity, the roots however differing somewhat with respect to one another. The similarity of the maximum velocities is borne out by the fact that the largest fibers are usually about 20 μ in diameter. On the other hand no regular morphological evidence for faster fibers in the sensory root appears. The larger fibers in the eighth pair are sensory but the reverse is true in the seventh. But, inasmuch as there is a variation in the relationship as recorded in the series of experiments quoted, these few analyses cannot be considered out of accord with the earlier observations. Among the observations in the same paper is an estimation of the range of fiber velocities in the motor root, derived from experimental data. The range was stated to be between 42 and 29 m.p.s. These values are in accord with the distribution curves if we assume that what was recorded in the experiments was the beginning and end of the main mass of the fibers, the actual total range must be larger.

Sciatic nerve. The nerve which has been most studied by us in the last few years and about which the most data have been collected, is the sciatic nerve of the bullfrog. More comparisons can, therefore, be made between reconstructions of this nerve and various characteristics of the action potential wave than are possible for any other nerve. An analysis of a part of the nerve for fibers above 5 μ gives the distribution curve recorded in figure 10, which shows again the three groups of fibers seen in the peroneal nerve. Reconstructions were made at 82 and 31 mm. of conduction (fig. 11) which may be compared with the potentials published in figure 2, 1924. The agreement is amazingly excellent, the differences being hardly greater than those between the two nerves of figures 2 and 3 in the same paper. The positions of the α and β crests coincide.

Fig. 6. Distribution curve of all the fibers (377) in the peroneal nerve of the bullfrog.

Fig. 7. °°°°° Reconstruction of peroneal action potential at 146 mm. --------- Corresponding recorded potential.

Fig. 8. Distribution curve of the VIII*th* pair of spinal roots of the bullfrog, (partial analysis). *a*. motor root (350 fibers, about $\frac{1}{4}$ total). *b*. sensory root (396 fibers).

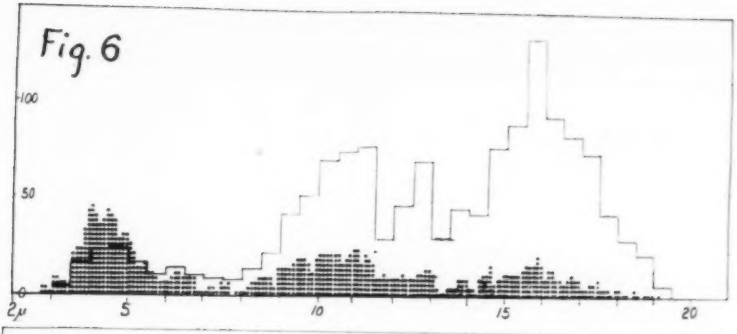


Fig. 6

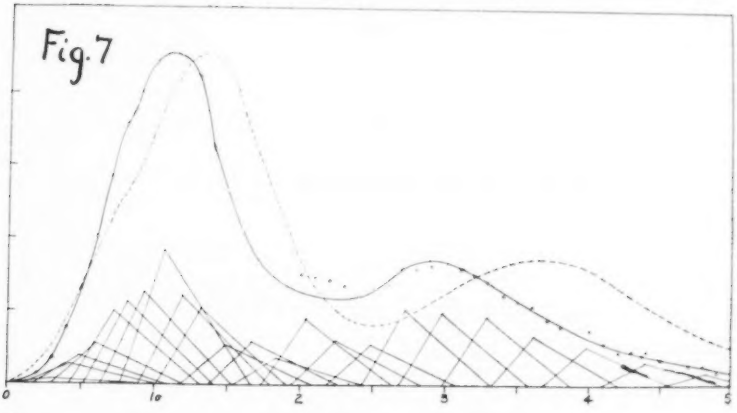


Fig. 7

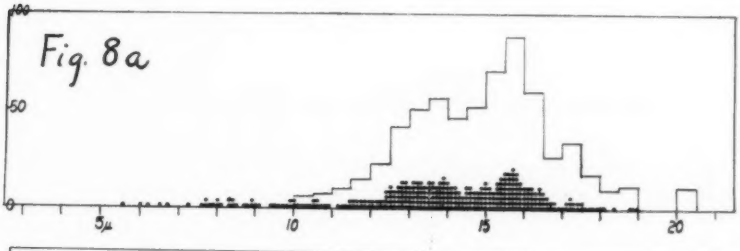


Fig. 8a

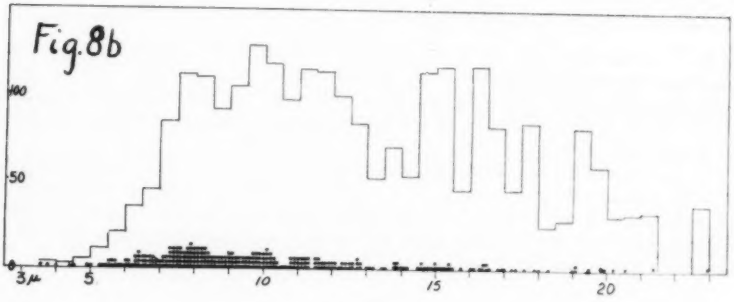


Fig. 8b

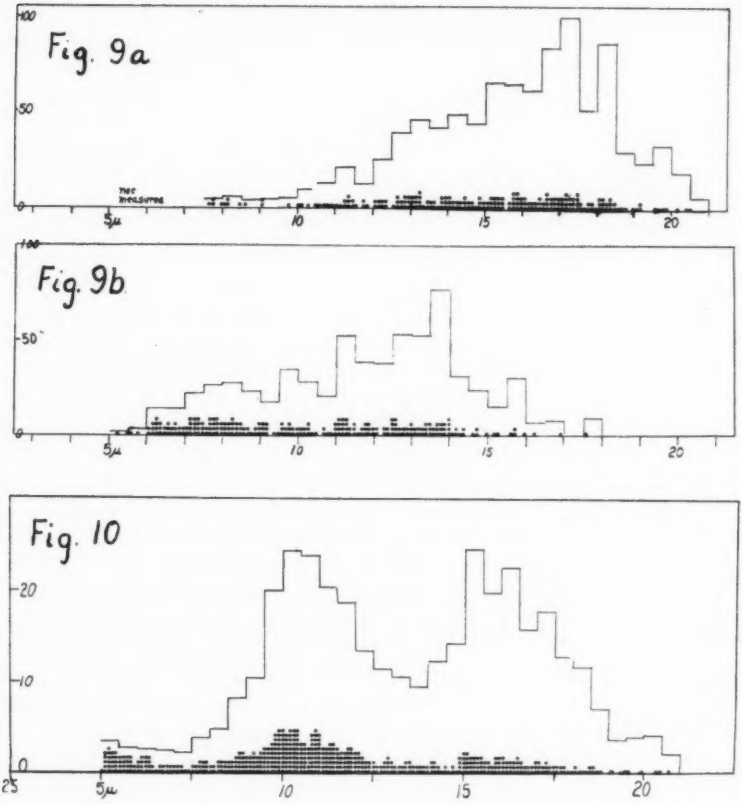


Fig. 9. VII*th* pair of roots (partial analysis). *a*. motor (194 fibers). *b*. sensory (287 fibers).

Fig. 10. Distribution curve for the sciatic nerve of the bullfrog (partial analysis, 514 fibers).

Fig. 11. Reconstruction of the action potential of the sciatic nerve of the bullfrog. ●●●●, at 82 mm. of conduction. xxxxxx, at 31 mm. of conduction.

Inset: duration of the rising phases at the distances indicated on abscissae. xxxxxx, from reconstructions. ●●●●, experimental data from table 2, 1925, lxxiii, 620, this Journal.

Fig. 12. Reconstruction of the maximum potentials at various distances of conduction. xxxxxx, whole nerve active. ○○○○, activity only in the α group. ●●●●, recorded maximum potentials taken from an experiment especially performed for the purpose. ++++, points on a curve of maximum potentials of the α fibers only (6/19/24).

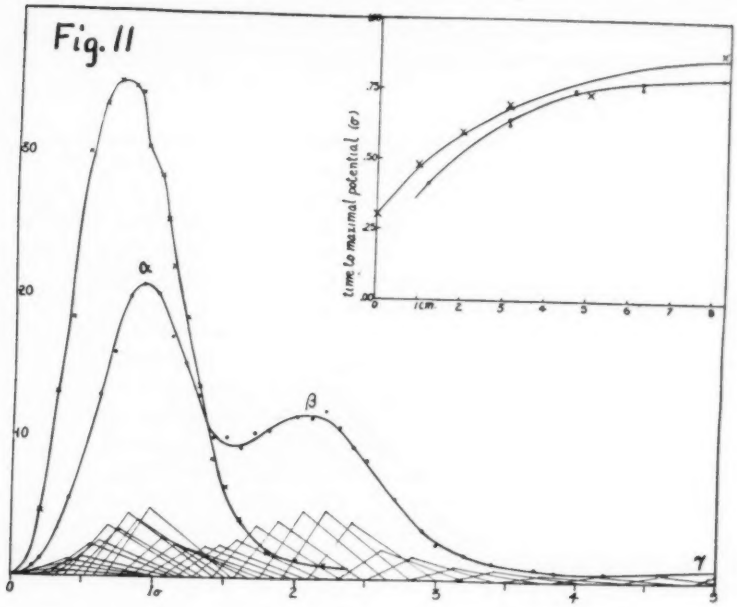


Fig. 11
α
β
γ
time to maximal potential (σ)
1cm.

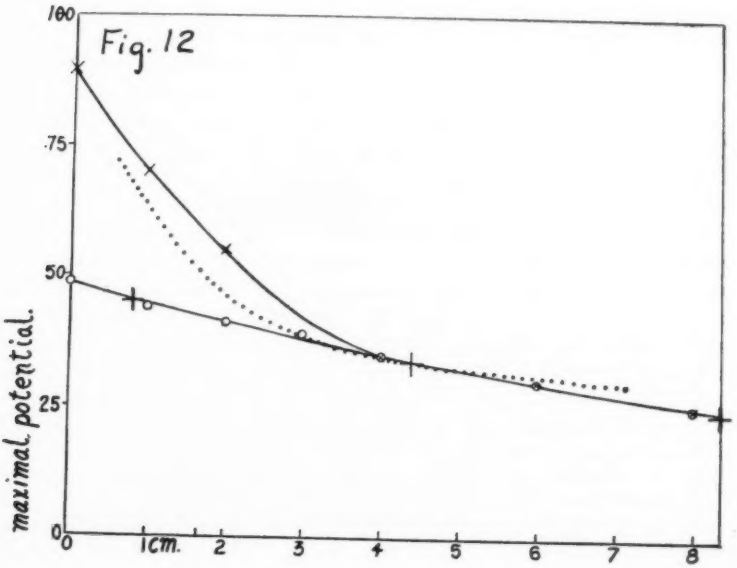


Fig. 12
maximal potential.
1cm.

Also a trace of the γ wave shows in the reconstruction. It begins about 0.5 σ later than the one in figure 2b, but a reference to the original figure in 2a shows that the position of this point in the line is not one to give accurate readings.

In addition to the reconstructions reproduced, others were made at various distances of conduction, so that the behavior of the predicted curves could be compared with the real ones. Taking first the duration of the phase of rising potential (inset, fig. 11), the theoretical upper curve has the same general shape as the lower curve obtained by experiment. They both start with a steep slope. The theoretical durations however are around 0.07 σ longer than the real ones and at 8 cm. the slope of the reconstructed curve is slightly greater. The reason for this will appear after we examine the maximum potentials at the various distances. These are recorded in figure 12. The upper solid line is the reconstruction for the whole nerve; it should be compared with the dotted line experimentally determined. The agreement is good enough to pass without comment. At the longer distances the slope of the real curve is less steep so that it comes to lie above the theoretical. This relationship corresponds with the slightly longer rising phases found at these distances in the reconstructions, and must be attributed to a slightly slower mutual separation of fibers than that postulated. The lower solid line is the reconstructed maximum potentials of the α fibers only. On this curve are placed as + signs data from an experiment in which these values were determined. The coincidence of the potentials with the theoretical, when plotted on the same scale as the latter, is exact. The two theoretical curves of the maximal potentials obtained when the whole nerve is active and when the α fibers only are active, deviate in figure 12, at about 4 cm. This is the position of deviation in fact; it merely means that beyond this point the large mass of fibers which makes up the β group is so far behind, that it does not enter into the maximum value at all; therefore, beyond 4 cm. the maximum is the same in the two cases.

Green frog nerve (Rana pipiens). The only green frog nerve that has been examined is the peroneal. Its analysis (fig. 13) gives a distribution which on reconstruction gives a β wave somewhat earlier than the real one, just as occurred in the bullfrog. The principal interest in this curve lies in the range of fiber sizes. The largest fiber is 13.9 μ in size, while the largest

Fig. 13. Distribution curve of approximately two-thirds of the fibers of the peroneal nerve of the green frog (377 fibers).

Fig. 14. Distribution of fibers in the dog phrenic. Partial analysis; 637 fibers which is a little over one-third.

Fig. 15 Distribution curves. *a.* phrenic (169 fibers, analysis partial). *b.* saphenous (358 fibers, analysis partial). Inset: reduced reconstructions of the phrenic at the distances of conduction of 1, 2, 4, 8 and 10 cm.

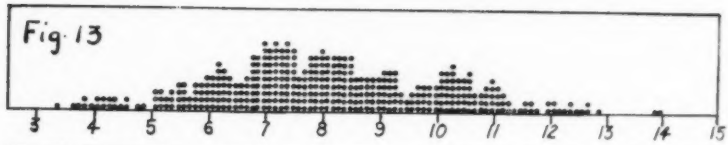


Fig. 13
3
4
5
6
7
8
9
10
11
12
13
14
15

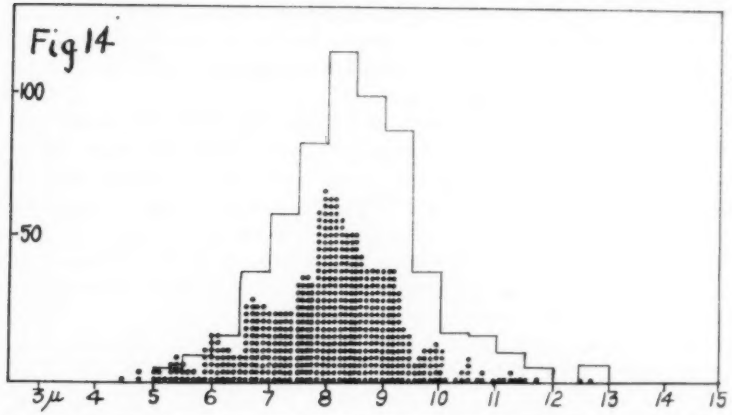


Fig 14
100
50
3μ
4
5
6
7
8
9
10
11
12
13
14
15

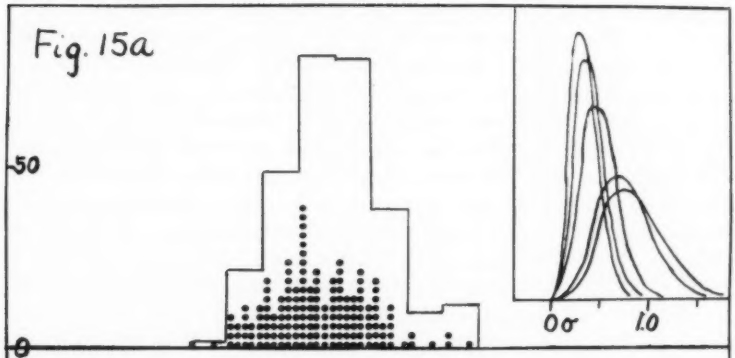


Fig. 15a
50
0
0σ
1.0

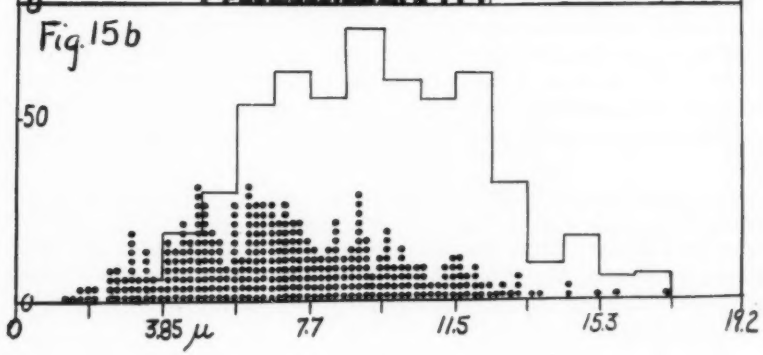


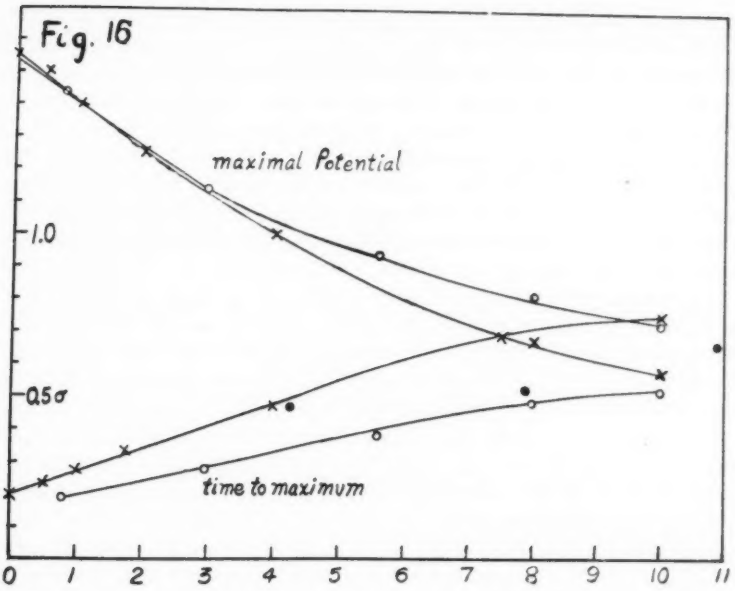
Fig. 15b
50
0
0
3.85 μ
7.7
11.5
15.3
19.2

fiber in the bullfrog nerve (fig. 6) was 19.2 μ. Ever since we have been working on bullfrogs we have known that its fastest fibers are much faster than those in the green frog, without having any explanation of why this should be so. It now appears that this is connected with the size of fibers. The recorded velocities were 31.8 m.p.s. and 43.2 m.p.s. respectively; 19.2/13.9 of 31.8 is 43.9, therefore size for size the nerve fibers in the two species seem to have the same velocity.

MAMMALIAN NERVE. The phrenic and saphenous nerves of the dog may be taken as two good examples of mammalian nerves giving action potentials of different forms. Three phrenic nerves have been analysed and two saphenous nerves. Of the former two are reproduced (figs. 14 and 15) and of the latter, one (fig. 15). The phrenic nerves have a single peak at 8 μ with the number of fibers dropping off to either side as occurs in a motor root, the total range being between 5 and 12 μ, the very small sympathetic fibers in the nerves not having been measured.

While the figure 14 analysis is more complete, it does not deviate from that in figure 15. As the latter was the first one made, it happens that the data have been examined in more detail. In the first place the data, as would be expected, give a perfectly smooth reconstruction which corresponds to the smooth action potential. Reconstructions were made up to distances of 10 cm., and the decrease in the maximal potential and the increase in the time to maximum were compared with experimental data in figure 16. Small size redrawings of the large size originals appear in the inset of figure 15. The curve of maximal potentials from the reconstructions is plotted to the scale of the recorded potentials so that the curves will coincide at the start; the durations of the rising phases are absolute values and are plotted as determined. The general slopes of the curves are alike. The deviation of the curves of maximal potentials is in such a direction that the reconstructed curve falls off more rapidly than the one from the nerve. Corresponding to this the time to maximum tends to increase slightly more rapidly in the former than in the latter. The explanation of this may be again that the axon potentials actually become mutually separated at a slightly slower rate than that postulated, though other factors can enter. Times to maximum differing less from the theoretical curve have been recorded, for instance in the experiment of 5/16/23, published in 1924, points from which are inserted into the figure as black dots.

The saphenous nerve differs from the phrenic in the large number of fibers below 6 μ, and in the possession of a few larger fibers. As the saphenous is purely sensory it follows that many of its functions are subserved by small fibers, such as do not go to a muscle at all. The muscle sense must therefore, as has been suggested by Sherrington, be carried by relatively large fibers.



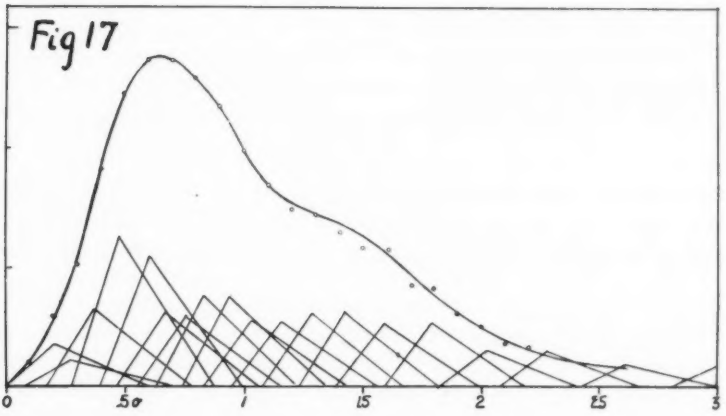


Fig. 16. Analysis of reconstructions of the action potential in the phrenic nerve of the dog made from the data in figure 15. XXXX, reconstructions. ○○○○, data from recorded action potentials of the phrenic of 2/14/24. ●●●●, data from recorded action potentials of the phrenic of 5/16/23 (published 1924).

Fig. 17. Reconstruction of the saphenous action potential at 95 mm., for comparison with that of experiment 12/16/22 (1924, p. 653, fig. 13).

In an experiment of 12/16/22, the velocity of conduction in the saphenous nerve was found to be 83 m.p.s. In the reconstruction of the saphenous action potential this velocity was assigned to the 15 μ group. This ignored the two fastest fibers and it must be admitted that this was done to obtain a better result. The two fibers omitted, however, were larger than any appearing in the other saphenous nerve examined. The resulting curve (fig. 17) bears a definite resemblance to the real action potential. The β wave is perhaps somewhat less prominent but it is definitely present, a point which is not so obvious at a first glance at the distribution curve.

The chorda-tympani and the lingual nerve of the dog. A statement of Heidenhain's that the chorda-tympani is made up almost entirely of 4 μ fibers led us to try to identify the velocity of fibers of this size, which could be observed both before and after entrance into the lingual. The lingual was dissected out and followed back to the point where the chorda-tympani entered it from the facial. The chorda was then traced to its point of emergence from the Glaserian fissure, excised and mounted in a moist chamber at 37°. By distal stimulation a lead could be taken from the chorda-lingual just before the chorda branch, then by a three millimeter shift the lead could be moved to the latter; when this procedure was followed a small wave appeared on the falling phase of the chorda-lingual action potential which was identified because it turned off into the chorda to become its action potential (fig. 18). A secondary wave was thus associated with a definite group of fibers, but the histological picture showed that, while 4 μ is the maximum of the distribution curve of the chorda fibers, there are many fibers on each side of this position extending from 1.5 μ up to 7 or 8 μ. The recorded velocities of the lingual fibers and the chorda fibers were respectively 69.1 and 36.4 m.p.s.; $13.5/8.2 \times 36.4$ is 60.0, a value considerably smaller than 69.1, which would indicate faster conduction in the slow fibers than that predicted from a strictly linear relationship. However, if the assumption be made that the potential effect of the two largest fibers of the chorda would be missed in an action potential as small as that of the chorda, then $13.5/7.25 \times 36.4 = 67.8$, which is in much better accord with the actual lingual velocity. While this experiment is in accord with the general thesis, it did not yield the decisive data which were hoped for.

Comparison of frog and mammalian nerve. We have seen that the difference between the velocities of the sciatic nerve fibers of the bullfrog and of the green frog can be explained by the difference in the sizes of the fibers. This is an incentive to examine what data are available on mammalian nerve, to see if, when allowance is made for the difference in temperature, the velocity in mammalian fibers may not be calculated from

that in the frog fibers. In making the comparison, fibers of the same size must be considered. The present state of the knowledge of the effect of temperature upon nerve is not sufficiently good for one to employ the temperature coefficient with any feeling of security. In general the coefficients are found to lie between 1.5 and 2.0. Lucas found for frog nerve an average of 1.79, Ganter 1.75, Broemser 1.79 for nerves kept a short period at the temperatures examined. Snyder reports higher values, but his average between 20° and 30° is 1.5. He gives a value of 2.6 between 10° and 20°; however, there are much lower coefficients to be calculated from his data. As Broemser has pointed out, the coefficient depends on

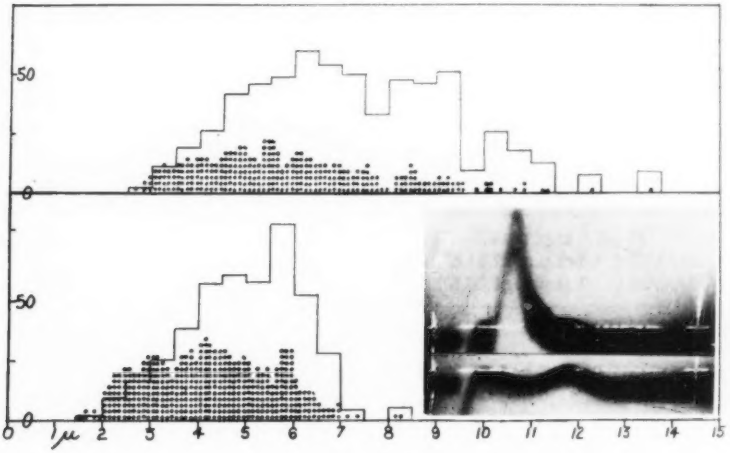


Fig. 18. Distribution curves of the chorda-lingual of the dog (upper) and the chorda-tympani (lower); partial analyses.

Inset: action potentials of chorda-lingual (upper) and chorda-tympani (lower); reproduction × 0.7. Movement of the chorda-lingual figure on the screen has caused it to make three impressions.

the previous condition of the nerve, and after conservation in Ringer's solution for 40 minutes before the determinations, he obtained values as low as 1.15 thus recalling the contention of Weiss that temperature is without effect. With the coefficient in frogs depending upon the temperatures from which it is calculated, upon the duration at those temperatures and upon the previous temperatures, selection of one for comparison with mammalian nerve is bound to be a purely arbitrary procedure. What is really done is to see what would happen in a mammalian nerve if the local reaction velocity in the latter can be compared with frog nerve by such a constant.

A start was made by comparing the green-frog sciatic and the phrenic nerve of the dog. The velocities of three frog nerves, whose temperatures at the time of observation were known, were selected from table 1, 1924. In figure 13 of this paper, which is the only analysis we have of a green frog nerve trunk, the largest fiber is 13.75 μ. In three phrenic nerves of the dog the largest fibers were found to be respectively 12.1, 12.3 and 12.7 μ (ave. 12.4 μ). From the velocities of the fastest fibers of a green frog the velocity of a 12.4 μ fiber was computed by the ratio of the diameters; then this velocity was recalculated for 37°. In order to cover the possibilities this was done for three values of Q_{10}: 2, 1.7 and 1.5. The calculation was easily made graphically with sufficient accuracy. The averages for the three fibers at the three coefficients were then determined and recorded in table 1.

TABLE 1

Velocities of conduction in green frog nerve calculated at 37°

NERVE SIZE (μ) 12.4	VELOCITY M.P.S.		
	$Q_{10} = 2$	$Q_{10} = 1.7$	$Q_{10} = 1.5$
11/18/22 (35.5 m.p.s. at 24.5°)	76.5	62.5	52.5
11/20/22 (29.8 m.p.s. at 21.6°)	77.0	60.5	50.0
11/22/22 (33.3 m.p.s. at 23.3°)	76.5	62.3	52.3
Average	76.7	61.4	51.6
If 17	105	84	71
If 14.4	89	71	60
At 13.5	84	67	56
At 8.2	51	40.6	34

In general the phrenic nerve has given quite variable velocities of conduction. One source of error, not realized in the earlier determinations, is that the nerve has a tendency to curl up in its sheath so that the measurements of the length may be inaccurate. In table 1, 1924, values of 50, 52.9 and 73.5 m.p.s. are given. A recent determination gave 61.5 m.p.s. (1/7/26). An average of the former, or the latter, agrees very well with the calculated velocity at 37° for the green frog using $Q_{10} = 1.7$. This value happens to be close to the average values of Q_{10} cited above.

In two saphenous nerves of the dog (1924) the velocities were respectively 83.3 and 71.5 m.p.s. In the two saphenous nerves which have been analysed for size of fibers the largest fibers were found to be 17 μ and 14.4 μ respectively. Accordingly, the velocities green frog fibers of these sizes would have, were calculated for 37° (table 1). If $Q_{10} = 2$ the velocities would be too high to accord with those observed. At $Q_{10} = 1.7$

however the two velocities accord very well with the two nerves on which we have data. At 1.5 the calculated velocities become too slow.

The other mammalian nerves on which we have the necessary information are the lingual of the dog and the chorda tympani, whose largest fibers are 13.5 μ and 8.2 μ respectively, with velocities of 69.1 and 36.4 m.p.s. These are comparable to the values in the $Q_{10} = 1.7$ column of table 1, which gives for these sizes 67 and 40.6 m.p.s. One experiment was made directly on the conduction rates in mammalian nerve at room and body temperature. The nerve employed was the popliteal division of the sciatic of the rabbit. At 24° the velocity was 44.2 m.p.s.; at 38.7°, 87.6 m.p.s., which would give a Q_{10} of about 1.6. An examination of the rabbit nerve showed that the largest fibers were about 18 to 19 μ in size which must be compared with 20 to 21 μ in bullfrog nerve. Forty-four m.p.s. is an average rate in bullfrog nerve at room temperature. It is therefore somewhat faster than the average velocity in frog 18 to 19 μ fibers. However, it is not an impossible figure for the latter, as in exceptional cases we have obtained velocities which would indicate rates as fast as 44 m.p.s. in such fibers.

The analyses as far as they go are compatible with the generalization that conduction in nerve fibers from different species depends primarily upon the size of fiber and the temperature. To establish this point will require a more extensive and detailed study necessitating the proof of lack of exceptions.

Discussion. The foregoing data are believed to show that the important variable in nerve fiber construction, determining the velocity of conduction, is the size. The shape of the action potential wave, the appearance of secondary waves, the increase in time to maximum potential and the decrease in its value can be satisfactorily explained at least to a first order approximation by conduction velocities differing from one another as the ratio of the diameters of the fibers. Deviations from this assumption are not constant, the small fibers conducting more slowly than thus postulated in the two peroneal nerves recorded at their distal ends, and slightly less slowly than postulated in some other nerves examined. In general the evidence indicates that, if the small fibers deviate from the rule, their conduction is slightly faster than that predicted from the ratios of their diameters to the maximum diameter in the trunk.

SUMMARY

In this paper the relation between the activity of nerve, as revealed by its electrical sign, and its morphology is studied.

In various nerves the fibers were measured and the results plotted so as to reveal graphically the number and area of the fibers at each size.

Theoretical reconstructions were made of the action potential waves

making use of the following observations and assumptions which are explained in the text.

a. In a given nerve trunk the duration of the axon action potential is the same in fibers of all velocities.

b. The wave form is assumed to be triangular but its dimensions are chosen so as to subtend an area equal to the recorded axon potential area, as near as this can be determined. For this purpose, in mammalian nerve the rising phase is taken as 0.2 σ and the duration as 0.6 σ. For frog nerve the corresponding values are 0.3 σ and 0.9 σ.

c. The rate of propagation is uniform throughout the fiber.

d. The maximum potential developed is the same in all fibers. The fiber would then affect the recording instrument as the internal resistance (cross-section) of the fiber.

The reconstructions closely resemble the actually recorded potential waves, if the velocities of conduction in the axons are assumed to vary directly as the diameters of the axons. *We therefore conclude that at least to a first approximation the velocity in a fiber is determined by the diameter.*

It is possible by a histological examination of a nerve trunk to predict with some accuracy the form of the action potential it will yield. The reconstructed action potential wave behaves with regard to form, changes of form with conduction (such as increase in the time to maximal potential and decrease of the size of this maximal potential), and appearance of secondary waves, in the same manner as does the corresponding action potential as recorded.

The distribution curves of the spinal roots are just as different as the action potential waves. The total range of fiber sizes in the two roots is nearly the same, but the peak of the area of the fibers comes at about 15 μ in the motor root, while in the sensory root the peak comes at 8 to 9 μ, with a secondary peak at 14 to 15 μ. The single peak in the motor root corresponds with its simple action potential curve. The two peaks in the sensory root on the other hand correspond with the two waves in its compound action potential. The second or β wave is due to fibers below about 12 μ in size. The area of this group is larger than that of the group of α fibers and corresponding to this, the area of the β group in the recorded potential is also larger; but the height is lower than that of the α wave due to the greater mutual spread of the smaller fibers on conduction.

In the sciatic and peroneal nerves the fibers appear in three groups. It is this unequal distribution which is responsible for the α, β and γ waves.

The velocity of conduction in the green frog sciatic is less than in that of the bullfrog at the same temperature; corresponding to this, the fibers in the former are smaller.

The distribution curves of the phrenic and saphenous nerves of the dog correspond to their widely different action potentials. As in the sensory

root the β wave in the latter nerve is due to the large mass of small fibers in its composition.

The chorda-tympani fibers in the chorda-lingual nerve, producing a secondary potential wave, may be identified by tracing them into the chorda before its union with the lingual. Their size is then found to correspond with the velocity of the wave.

The available data are analysed for the degree of relationship which may be inferred between mammalian and frog fibers of the same size, when the temperature coefficient is taken into consideration.

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THE FATE OF DIHYDROXYACETONE IN THE ANIMAL BODY[1]

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The supposed intermediary degradation products of glucose in the animal organism have been the subject of considerable investigation and much more ratiocination. The premises usually have been: first, the existence of a substance with such chemical characteristics as do not make it impossible to be derived from glucose either in vitro or in vivo; second, its existence or supposed existence in animal or vegetable life; third, the demonstration that it is a glycogen former in the animal body; fourth, the demonstration that the substance is quantitatively or nearly quantitatively excreted as glucose by the phloridzinized or depancreatized animal. It may be pointed out that such premises do not necessarily lead to the conclusion that the substance is normally formed in the course of the katabolism of glucose and thereafter transformed directly or indirectly to carbon dioxide and water. Lactose, for instance, fulfils all of the above premises but can hardly be considered in the normal path of degradation of glucose. With regard to three such substances the statement of Dakin (1) in his review of the carbohydrates may be quoted: "It appears to the writer that at the present time it is impossible to reach a definite decision as to the rôle of glyceric aldehyde, dihydroxyacetone, and pyruvic aldehyde in glucose metabolism." It appeared to us possible to investigate the question of the intermediary metabolism of glucose (in its strict sense) with regard to the rôle of dihydroxyacetone in other ways. The earlier results have already been reported in a series of papers.

In the first paper (2) a method for the quantitative determination of dihydroxyacetone in the presence of glucose was described. By this method it was possible to show that, if present at all, the quantity of dihydroxyacetone in normal or diabetic blood must be exceedingly minute, and that administration of the triose by mouth, either to normal individuals or diabetic patients (2), (3), causes its appearance in the blood stream in quite considerable amounts. In the second paper (3) it was shown that the hypoglycemic symptoms produced by insulin in man and laboratory

[1] The expenses of this investigation were defrayed by a grant given for research on insulin by the Carnegie Corporation of New York.

animals are removed and the blood sugar caused to rise to well above the hypoglycemic level by the administration of dihydroxyacetone, and it was pointed out that since this is a specific property of glucose, and to a less extent of mannose, the dihydroxyacetone must be converted into one or other of these sugars. In the following paper (4) we show when dihydroxyacetone is given to depancreatized dogs deprived of insulin that the rise in blood sugar coincides with a fall in the dihydroxyacetone in the blood, that such animals excrete the triose quantitatively as glucose and that the respiratory quotient is not affected. We have also shown (5) that animals receiving insulin on a constant diet containing glucose in excess (so that there is glycosuria) do not metabolize dihydroxyacetone any more efficiently than glucose, either when it is substituted for, or is added along with the sugar in the diet. This evidence appears to indicate that dihydroxyacetone is not a katabolite of glucose but rather an anabolite and that it plays no part in the katabolism of glucose under normal conditions.

The purpose of the present paper is to present further evidence as to the site of transformation of dihydroxyacetone into glucose and also to examine the metabolism of the triose in blood and muscle tissue. It illustrates the method by which we have planned to investigate whether a substance is or is not an intermediary in the combustion of glucose in the animal body.

Embden, Schmidt and Wittenberg (6) perfused the surviving livers of dogs with blood containing dihydroxyacetone and found that glucose was formed in the perfusate. Mostkowski (7) fed the triose to fasting hens and observed an increase in the glycogen content of the liver, as compared with that in fasting control animals. One might naturally infer from this evidence that the liver is the site of a synthesis of glucose from dihydroxyacetone, but synthesis elsewhere in the body is not excluded. This could only be done by showing that no transformation of triose into glucose takes place when the liver is excluded from the circulation.

Mann (8) has shown that the blood sugar of dogs falls when the liver is excised and the clinical syndrome of hypoglycemia develops. Immediate improvement of the dog's condition is accomplished by administration of glucose. Other profound metabolic changes are induced by hepatectomy so that the animal, although it be adequately supplied with glucose, survives, as a rule, only from twelve to fifteen hours, and at most for thirty-five hours. It appeared to us probable that the fate of dihydroxyacetone in the body could be studied by observing its behavior when administered to liverless animals. During the course of this investigation a paper appeared recording, among other data, the results of administration of dihydroxyacetone for a period of forty minutes to an eviscerated, decerebrated cat by a continuous injection pump at the rate of 0.15 gram

per kilogram body weight per hour. While the blood sugar fell the dihydroxyacetone content of the blood showed no material change. It is inferred that the triose was utilized immediately and at a greater rate than glucose (Kermack, Lambie and Slater, 9).

Meyerhof, Lohman and Meier (10) state that dihydroxyacetone has no influence on the respiratory metabolism of excised amphibian muscle; methylglyoxal increases it slightly; glyceric aldehyde raises it about 30 per cent; and pyruvic acid very much more.

EXPERIMENTAL. Dogs were used in these experiments. The technique varied according to the particular problem we wished to study. Typical protocols are submitted, followed by comment. The analytical methods used were: for blood sugar, the method of Folin and Wu (11); for dihydroxyacetone, the method recently described by one of us (2). Wherever dihydroxyacetone was found in the blood the blood sugar readings have been corrected by allowing for the reduction due to the triose on the basis that the reduction of Folin-Wu copper tartrate reagent by the triose is 79 per cent of that produced by an equal amount of glucose. A correction has also been applied for the control blank determinations where necessary.

PROTOCOL. Dog 1—male terrier, black and tan markings; 10.5 kgm. Anesthetized with ether 2:10 p.m. Mid line incision. Ligated hepatic artery and branches in the fissure. Eck fistula established between vena cava and portal vein. Ligated portal vein proximal to last branch. Operation completed and dog conscious at 3:00 p.m. Animal walked about laboratory.

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
4:20	Blood sugar 1 taken	0.072	0
4:48	Blood sugar 2 taken	0.066	0
5:30	Dog rather drowsy		
5:45	Blood sugar 3 taken	0.061	0
6:44	Semi-conscious; legs stiff; muscle twitchings		
6:48	Blood sugar 4 taken	0.040	0
6:56	4–5 grams dihydroxyacetone given intravenously in 20 cc. physiological saline		
7:02	Blood sugar 5 taken	0.028	0.065
7:17	Blood sugar 6 taken	0.040	0.039
7:32	Blood sugar 7 taken	0.050	0.019
7:35	Animal drowsy but can walk		
7:40	Blood sugar 8 taken	0.050	0.009
7:50	Walked well; drank water		
8:00	Blood sugar 9 taken	0.060	0.000
8:15	Drowsy, can be roused		
8:45	Semi-conscious		
8:55	Twitching; later, convulsions		
9:12	5 grams dihydroxyacetone in 20 cc. saline intravenously		

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
9:15	Blood sugar 10 taken	0 032	0 038
9:37	Blood sugar 11 taken	0 033	0 024
10:15	Blood sugar 12 taken	0 047	0 004
10:45	Animal in good condition. Killed and necropsy performed. No hemorrhage. Ligatures properly placed and still tight		

PROTOCOL. Dog 2—Irish terrier. 15 kgm. Anesthetized with ether at 2:15 p.m. Operation as in preceding protocol. Dog conscious at 3:10 p.m. Walking about room at 3:30 p.m.

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
3:38	Blood sugar 1	0 083	0
4:15	Blood sugar 2	0 074	0
5:15	Blood sugar 3	0 063	0
6:15	Blood sugar 4	0 056	0
6:30	Animal still in excellent condition		
7:35	Blood sugar 5	0 041	0
	Animal stupid; walks with urging		
8:10	Blood sugar 6	0 041	0
	Lying down		
9:38	Blood sugar 7	0 041	0
	Semi-conscious; lying relaxed		
10:14	10 grams dihydroxyacetone in 34 cc. saline given intravenously		
10:52	Blood sugar 8	0 057	0 055
	Slight improvement		
11:40	Blood sugar 9	0 047	0 019
12:00	Blood sugar 10	0 043	0 009
12:05	Glucose given intravenously. No improvement. Died at 12:30 in hypoglycemic convulsion. P.M. no hemorrhage. Ligatures tight		

PROTOCOL. Dog 3—male collie; 18 kgm. Ether anesthesia at 10:45 a.m. Operation same as before. Operation completed, animal conscious at 11:45 a.m. An excellent recovery.

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
1:20	Blood sugar 1	0 143	0
3:30	Blood sugar 2	0 099	0
6:12	Blood sugar 3	0 090	0

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
7:15	Blood sugar 4	0.068	0
	Up to this time has been active. Cannot get up. Wags tail		
8:15	Blood sugar 5	0.068	0
9:15	Blood sugar 6	0.026	0
	Semi-conscious		
9:50	20 grams dihydroxyacetone in 60 cc. saline intravenously		
10:14	Quite unconscious		
10:25	Blood sugar 7	0.040	0.051
10:50	Blood sugar 8	0.050	0.021
	Animal quite unconscious; has shown no improvement		
11:10	Blood sugar 9	0.053	0.009
11:13	5 grams glucose in 15 cc. saline		
11:18	Walking spontaneously; responds to call		
11:30	Blood sugar 10	0.127	0.003
12:00	Animal unsteady on feet but roaming around room		
12:05	Suddenly dropped down		
12:09	Died. Post mortem revealed no cause for sudden death		

COMMENT. The first three protocols exemplify the results obtained by a so-called "functional hepatectomy." The animal very slowly goes into a hypoglycemic state. With the administration of dihydroxyacetone there is slight but very slow and incomplete recovery. Indeed at times we were quite uncertain from the clinical symptoms whether any improvement was taking place, though the chemical analyses show a rise in the glucose and, at the same time, a fall in the dihydroxyacetone in the blood. At no time was sufficient glucose produced to raise the blood sugar out of hypoglycemic levels. In contrast to this one finds immediate improvement when glucose is administered (dog 3), even though the animal had been hypoglycemic for two hours. These results, both in the clinical and the chemical effects, are markedly in contrast with those obtained when dihydroxyacetone is given during insulin hypoglycemia in man and in rabbits. They also stand in contrast to the rapid production of glucose which occurs when depancreatized animals are fed with dihydroxyacetone (4). On the other hand, even considering that the metabolism of the animal was probably far below that of a normal animal, the dihydroxyacetone did not disappear from the blood as fast as would be expected were the substance immediately available for the provision of energy, i.e., were an intermediary katabolite of glucose.

PROTOCOL. Dog 4—male collie; 10 kgm. Under ether anesthesia the vena cava was subtotally ligated eight days previously. Ether anesthesia commenced at 11:25. Ligation of celiac axis, superior and inferior mesenteric vessels at their roots. Rectum divided between ligatures. Vena cava and esophagus clamped off below the diaphragm. Excision of liver and alimentary tract. Abdomen packed with towels moistened with physiological saline. Operation complete at 12:05. Good recovery. Animal walked around laboratory.

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
1:30	Lying down		
2:30	Blood sugar 1	0 048	0
2:35	Twitchings. Mild convulsions		
3:10	Blood sugar 2	0 040	0
3:12	6 grams dihydroxyacetone intravenously in 20 cc. physiological saline		
3:30	Blood sugar 3	0 031	0 129
	Animal unimproved		
3:50	Blood sugar 4	0 033	0 124
	Unimproved; given 5 grams glucose intravenously; regained consciousness		
4:20	Walking for a time. Gradually became weaker		
6:30	Destroyed. Autopsy negative		

These results, then, indicate that, when functional hepatectomy is performed, there is a decided decrease in the efficiency of the conversion of dihydroxyacetone to glucose, and they make it certain that the liver is a source and probably the most important source of this conversion. On the other hand, they lend no support to the view that dihydroxyacetone is used as such by the animal when the main circulation through the liver is occluded. There remains to explain why after dihydroxyacetone was administered in these experiments a small but definite rise occurred in the blood sugar accompanied by a gradual disappearance of the triose from the blood. No doubt the explanation is that in the "functionally hepatectomized" animal the liver still continues active to a certain extent. In 1885 Stern noted (12), what has since been confirmed many times, that there is an important collateral circulation to the liver through the diaphragm. Recently Rich (13) has most clearly confirmed this. Occasionally one finds even very large vessels passing from the liver to the diaphragm and after ligating the vena cava and the portal vein so that the lumina are about four-fifths occluded we have been able to observe the development of an extensive collateral circulation through these vessels. In the acute experiment, after tying off the portal vein and hepatic artery and taking particular care to include all aberrant vessels these collaterals doubtless carry an increased supply of blood to the liver and this could

account for the small transformation of dihydroxyacetone into glucose. Incidentally it may be remarked that unless the anatomical conditions are radically different in the cat, this collateral circulation constitutes a defect in the Lambie type (14) of abdominal evisceration, as used by Kermack, Lambie and Slater (9), and is sufficient to account for the results obtained by the workers even if the amount of dihydroxyacetone transfused had been many times that actually found in the preparation.

Another possible complicating factor in these experiments is that the dihydroxyacetone may have been excreted into the intestine, which was left in the preceding experiments, and by mixing with the alkaline contents have become polymerized into hexose which was then absorbed into the blood.

PROTOCOL. Dog 5—terrier, male; 8 kgm. Subtotal ligation of vena cava and pancreatectomy three weeks before. Treated with insulin and diet. Fasted without insulin for thirty-six hours. Ether anesthesia 9:30 a.m. Excision of liver and alimentary tract as before. Renal vessels ligated. 10:10 operation completed. Animal made an excellent recovery.

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
12:20	Semi-conscious		
12:30	Blood sugar 1	0.050	0
	Convulsions		
12:30	10 grams dihydroxyacetone in 30 cc. water intravenously		
12:45	Blood sugar 2	0.041	0.211
	Convulsions		
1:00	Blood sugar 3	0.040	0.180
	Animal in desperate condition. Given 0.75 gram glucose intravenously. Much improvement		
1:15	Blood sugar 4	0.081	0.179
1:30	Blood sugar 5	0.075	0.180
1:43	Stopped breathing. Artificial respiration. Given 4 grams glucose intravenously. Improved; breathing spontaneously		
2:00	Blood sugar 6	0.263	0.180
2:40	Died. Autopsy: ligatures tight; nothing to vitiate experiment. Weight 8 kgm. without viscera		

COMMENT. *Dogs 4 and 5.* A preliminary subtotal ligation of the vena cava was carried out on dog 4 in order to increase the collateral circulation. After eight days the liver, spleen, pancreas and intestines were removed, the abdomen being packed with towels. Such a preparation lives several hours in good condition if the blood sugar level is maintained, but otherwise it rapidly succumbs. Administration of dihydroxy-

acetone intravenously to this animal after it had become hypoglycemic brought about no improvement in the clinical condition. The blood sugar continued to fall, and the concentration of dihydroxyacetone remained at the same level as immediately after the injection. Sufficient dihydroxyacetone was present in the blood to bring the blood sugar to

PROTOCOL. Dog 6—young female collie. Subtotal ligation of portal vein and vena cava eight days previously. Evisceration as before under ether anesthesia. Renal vessels ligated. 11:00 a.m.—12:00 noon. Animal made good recovery. Weight 10.5 kgm. after operation.

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
1:38	Convulsions		
1:45	Violent convulsions		
	Blood sugar 1	0 031	0
	Respiratory spasms. 0.75 gram glucose intravenously		
2:00	Animal unconscious. 1 gram left rectus femoris muscle taken for determination of dihydroxyacetone (T_1) and glucose		
2:35	Blood sugar 2	0 029	0
	Convulsions returning. 9 grams dihydroxyacetone given with 2 gram glucose intravenously		
3:10	Blood sugar 3	0 027	0 136
	Mucus cleared from throat		
3:30	Animal improved		
3:40	Blood sugar 4	0 014	0 127
3:50	Breathing irregularly. Given 20 grams glucose intravenously		
4:05	Breathing normally		
	Blood sugar 5	0 210	0 127
4:10	Mucus in throat. Trachea cleared. Instant improvement		
4:30	Blood sugar 6	0 236	0 130
4:53	Blood sugar 7	0 170	0 127
	Condition good but animal unconscious		
5:10	One gram muscle taken from right rectus femoris (T_2)		
5:25	Mucus in throat		
5:40	Died. Autopsy: no hemorrhage		

	GLUCOSE	DIHYDROXYACETONE
	per cent	*per cent*
Analysis of muscle:		
T_1	0 154	0
T_2	0 133	0 090
(Muscle rubbed through sieve)		

above the normal level had it been possible for the triose to be converted into glucose. The rapid removal of the hypoglycemic symptoms when a smaller quantity of glucose was administered stands in marked contrast to the results of administration of the triose. We may, therefore, conclude that the dihydroxyacetone was not converted into glucose nor used as a triose in the animal.

In protocol 5 the preparation of the animal was the same with the addition that the animal had been depancreatized three weeks previously and had been deprived of insulin for thirty-six hours so that the tissues might be regarded as free from active insulin. This procedure should increase the rate of utilization of intermediary katabolites of glucose by the tissues instead of which we find that the blood concentration of dihydroxyacetone, after the preliminary period of equilibration with the tissues, remained unchanged. In other respects the results are similar to those of experiment 4, though more striking since the period during

PROTOCOL. Dog 7—Female Boston bull. Subtotal ligation of portal vein and vena cava twelve days previously. Complete evisceration with ligation of renal vessels under ether anesthesia, 10:00–11:00 a.m. Section of gastrocnemius muscle taken for dihydroxyacetone (T_1). Good recovery. Weight after operation, 9.1 kgm.

TIME	REMARKS	BLOOD GLUCOSE	DIHYDROXYACETONE
		per cent	*per cent*
11:40	Blood sugar 1	0.10	0
11:45	3.5 grams glucose 9 grams dihydroxyacetone		
12:00	Blood sugar 2	0.205	0.167
12:10	Fully conscious		
12:35	Blood sugar 3	0.106	0.134
12:40	Convulsion (asphyxial?) 5 grams glucose given Throat cleared of mucus		
1:10	Blood sugar 4	0.304	0.121
1:35	Blood sugar 5 2 grams glucose intravenously. Asphyxial convulsions from mucus in throat	0.275	0.120
1:45	Animal unconscious. 2 grams gastrocnemius muscle taken (T_2)		
2:15	Animal allowed to die of respiratory failure. Postmortem examination: no hemorrhage		

	GLUCOSE	DIHYDROXYACETONE
	per cent	*per cent*
Analysis of muscle:		
T_1	0.387	0
T_2	0.308	0.078
(Microtome sections)		

which the triose content of the blood was followed was longer and the caloric requirement of the animal much greater. Again, in contrast to the results with dihydroxyacetone, marked improvement in both the clinical condition and the blood-sugar concentration occurred following the intravenous administration of glucose.

COMMENT. *Protocols 6 and 7.* The protocols 6 and 7 record the results of experiments on dogs in which both vena cava and portal vein had previously been subtotally ligated. An excellent collateral circulation is rapidly developed in such cases. Evisceration was performed as previously described and the renal vessels were ligated. These animals secreted a large amount of thick, ropy, mucous saliva and considerable difficulty was experienced in keeping their throats free from it. It seems by no means clear whether this is a coincidence or the result of tying the renal vessels. Dog 6 was allowed to become hypoglycemic. This was prevented in dog 7 by injecting sufficient glucose. The effects of administration of dihydroxyacetone were similar to those previously noted. In dog 6 the blood sugar (no. 4) apparenty fell to the extremely low value of 0.014 per cent, but there was insufficient filtrate for a duplicate analysis. In addition, analyses were made on the muscle tissue before the administration of the triose and at the close of the experiment. In dog 6 both the first and second portions of muscle were removed during hypoglycemic coma. From dog 7 the first section was taken during ether anesthesia; the second during asphyxial coma. The muscle from dog 6 was forced through a sieve to break it up; that from dog 7 was rapidly frozen with liquid CO_2 and cut into twenty-five micron sections by a microtome. Both were placed for ten minutes in water to permit diffusion to take place, then precipitated by the Folin-Wu tungstic acid process. It is realized that examination of these tissues alone is insufficient evidence on which to base exact calculations of the body content of dihydroxyacetone since different muscles will probably take it up in different amount. Calculations, after deduction of the blank, based on the assumption that the dihydroxyacetone is evenly distributed in 75 per cent of the animal, an assumption used by Bollmann, Mann and Magath (15) in their work on uric acid metabolism in hepatectomized dogs, yields values of 7.1 and 5.3 grams or 79 per cent and 59 per cent respectively of the total amounts injected. (If the blood triose values are used in a similar calculation the result is 10.0 and 8.2 grams respectively.) Considering the need for calories this amount of unchanged and unused dihydroxyacetone indicates that the triose cannot be in the normal path of degradation of glucose. Failure of the concentration of dihydroxyacetone in the blood to fall indicates that an equilibrium had been attained between concentrations of the triose in the blood and tissues.

DISCUSSION. The fall in blood sugar which ensues in dogs after anasto-

mosis of the portal vein with the vena cava and ligation of the hepatic artery may be partially restored by the administration of dihydroxyacetone intravenously. This is due to the conversion of some of the triose to glucose, but the process is very slow and unsatisfactory as is further evidenced by the tardy disappearance of the former from the blood. The result indicates not only that there can be no mechanism for effecting this change outside the liver, but also that dihydroxyacetone can not be used directly to any significant extent by the tissues. Reasons are given for our belief that functional hepatectomy of this type provides an unsatisfactory preparation on which to investigate the problem since the collateral circulation through the liver is sufficiently extensive to account for conversion of some of the triose to glucose. It seems to us probable that the experiment of transfusing an animal by continuous administration with small concentrations of dihydroxyacetone, as performed by Kermack, Lambie and Slater, reveals no increase in concentration of the triose in the blood because of its being transferred to the tissues. In the experiment as performed, the possibility always exists that the triose is converted to glucose by being carried to the liver by way of the collateral circulation to this viscus. Such small quantities of glucose as would be formed in this manner are, however, far below the utilization requirement of the animal for glucose, so that no increase in blood sugar would be noticeable, the glucose as it was formed being immediately oxidized or converted to glycogen by the tissues.

The second group of experiments was done on eviscerated animals, and they confirm the tentative conclusions that were drawn from the results of those of the first group. They also reveal a further fact of importance, namely, that although the blood sugar remains low following the intravenous administration of dihydroxyacetone the concentration of the triose itself in the blood falls to a level at which it remains and which is, presumably, in equilibrium with that in the tissues. Were the substance converted to glucose and used as such the blood sugar would be expected to rise, and the animal to recover from the symptoms of hypoglycemia. Furthermore, were the dihydroxyacetone directly oxidized by the tissues, as would be the case were it an intermediary degradation product of glucose, its concentration in the blood should show a continuous fall to satisfy the tissue requirements for calories. As neither of these changes occurs it may be concluded that the liver is the only place of conversion of the triose into hexose and that the triose is not utilizable as such by the tissues.

Evisceration of a depancreatized dog after the withdrawal of insulin should increase the rate of direct utilization of the triose and cause its rapid disappearance from the blood stream. The results of such an experiment, however, do not show any greater utilization than in the previous experiments.

The results of this investigation are in conformity with those of Mostkowski who found that additional glycogen was formed in the liver of fasting hens after feeding them with dihydroxyacetone, and with those of Meyerhof and his collaborators who found no utilization of the triose by excised amphibian muscle.

In light of these results it seemed probable that dihydroxyacetone should be recoverable from the tissues, particularly from the muscle tissue, and this we have succeeded in doing in such quantities as to furnish strong evidence that none of it can be oxidized when it disappears from the blood stream. These results are consistent with those of our previously recorded investigations on the metabolism of dihydroxyacetone in the animal body. Since they indicate that there is no utilization of the triose when the liver is removed from the circulation it seems improbable that dihydroxyacetone is an intermediary substance between glucose and its ultimate decomposition products. Since it has also been shown that the substance must be converted to glucose by the liver it is useless to expect that it can be metabolized with any greater efficiency by the diabetic patient than glucose itself.

CONCLUSIONS

Dihydroxyacetone is converted to glucose by the liver and used as such. In the absence of the liver this conversion does not take place, but when the triose is injected into a liverless animal part of it enters the tissues. The concentration of the triose in the blood under these conditions falls to a certain level which represents an equilibrium between blood and tissues notwithstanding severe hypoglycemia and a very considerable requirement for calories. The concentration of dihydroxyacetone which we actually found in the tissues at the end of the experiment, as calculated from the concentration of the triose in a muscle, is sufficient to account for a very large part of the triose administered. In conjunction with the results reported in previous papers it is concluded that dihydroxyacetone is not in the normal path of katabolism of glucose. Since, as our results clearly show, this substance must be converted to glucose as a preliminary step in its metabolism it is not possible that it can be metabolized by the diabetic patient more efficiently than glucose itself.

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ON THE METABOLISM OF DIHYDROXYACETONE IN PANCREATIC DIABETES[1]

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With the development of a method for the production of dihydroxyacetone on a commercial scale sufficient quantities of this substance have become available to test properly the hypothesis that this substance is formed as an intermediate step in the breakdown of carbohydrate in the animal body. The following facts have been offered in support of this view: First, the presence of dihydroxyacetone in the products of fermentation of hexoses by yeast (1); second, its influence in causing excess of glucose to appear in the nutrient fluid perfused through the excised liver (2); third, its conversion into glucose when administered to dogs under the influence of phlorhizin (3); and fourth, its non-toxic properties when given to animals in contrast to the decidedly toxic effect of the other related trioses, dextro- and laevo-glyceric aldehyde (4).

This indirect evidence is, however, far from convincing. For example, it is well known that the chemical processes taking place in plant life are not necessarily those obtaining in the animal, and the fact that it can form glucose under certain conditions in the animal body does not prove that it must be formed during the breakdown process. That it is non-toxic, whereas the other trioses are decidedly so, does not rule out the possibility that the other paths of chemical breakdown are possible, through the production of methyl glyoxal.

Our interest in this problem was aroused by the statements of Isaac and Adler (5) and Rabinowitch (6) that dihydroxyacetone could be utilized by the diabetic patient so as to produce energy and act as an antiketogenic substance. These workers have advocated that this substance should be added to the diet of diabetic patients instead of administering insulin to them. In testing this possibility on clinical material results were encountered which it is impossible to reconcile with these views. Since irregularities in the output of sugar and ketone bodies in diabetic patients, due to there still being a certain amount of insulin secretion from the

[1] The expenses of this investigation were defrayed by a grant given for researches on insulin by the Carnegie Corporation of New York.

pancreas, make the clinical observations of uncertain value, it was decided to repeat the observations on completely depancreatized dogs.

In a preliminary communication (7) we stated that dihydroxyacetone given by mouth to the fasted depancreatized dog is recovered quantitatively in the urine as glucose, while the respiratory quotient behaves in the same way as after giving equivalent amounts of glucose. Evidence has also been obtained by us that dihydroxyacetone when administered to normal, as well as to diabetic animals, is transformed into glucose. In neither type of animal does the blood contain any dihydroxyacetone; it appears when some is administered to the animal but soon disappears again. It is capable of removing the symptoms of insulin hypoglycemia, and we have found in such cases that the sugar rises in the blood *pari passu* with the disappearance of dihydroxyacetone from it. This along with the fact demonstrated by Herring, Irvine and Macleod (8) and by Noble and Macleod (9) that only glucose and very closely related hexoses (e.g., mannose) can act as efficient antidotes on insulin hypoglycemia, makes it almost certain that any antiketogenic influence of dihydroxyacetone must depend on its conversion into glucose. In the present paper we wish to present more fully the evidence on which these conclusions are based.

Since our preliminary communication two papers by Mason (10), (11) have appeared describing the effects produced on the total metabolism and the respiratory quotient in normal and diabetic individuals after receiving 25 to 50 grams of dihydroxyacetone by mouth. Both the respiratory quotient and the oxygen consumption rose to a greater extent after dihydroxyacetone than after glucose, and the blood sugar increased less. These results are confirmatory of those previously recorded by Rabinowitch (6), who found that the respiratory quotient rose from 0.706 to 0.817 in a diabetic patient following administration of 10 grams of dihydroxyacetone.

TECHNIQUE. Depancreatized dogs were used. In all cases the pancreatectomy was done some weeks or months previous to the observations, insulin being given meanwhile and the wound healed without infection. The animals were well nourished; some were even fat. Subsequent necropsy proved the pancreatectomy to be complete in all cases. For the observations the animals were placed in metabolism cages and all food was withdrawn, although water was allowed *ad libitum*. Insulin was withheld except for certain purposes, as noted later. The dog was catheterized and the cage washed down at a definite time each morning. All urine and washings were saved under toluol for analysis. When vomiting occurred the vomitus was saved separately if possible, otherwise it was collected with the urine. In these cases mucin was removed by precipitation with acetic acid and filtration before nitrogen estimations were carried out. After some days of fasting the animal was given weighed

amounts of dihydroxyacetone in solution by mouth at a time suitable for the particular experiment.

The urinary analyses were carried out as follows: nitrogen by a modified Kjeldahl method; total sugar by the Benedict method for glucose (12); total acetone bodies by Van Slyke's method (13); inorganic phosphates by Briggs' method (14); and dihydroxyacetone in blood by a method recently described (15) which, however, is not applicable to urine. Qualitative detection of dihydroxyacetone in urine was made by a more sensitive modification of the test proposed by Rabinowitch (6) by which a rough idea of the quantity could be obtained. We found estimation of the reducing power after fermentation of the urine to be an inaccurate measure of the dihydroxyacetone, since Bertrand has shown, contrary to what is usually stated, that dihydroxyacetone is fermented by yeast. We accordingly employed a method based on the difference between the polarimetric and the titrimetric measurement of the total sugar. This value is, if anything, too large, since any β-oxybutyric acid present reduced the polarimetric reading. As the amount of the latter substance was never very great, the error involved may be neglected. Blood sugar determinations were made by the method of Folin and Wu (16).

EXPERIMENTAL. We have adopted the plan of recording consecutively: First, the protocol; second, the urinary analyses; third, the blood analyses; fourth, the respiratory analyses. In some cases it was not convenient to obtain all of these data and in such cases the corresponding table is omitted. Comment on the results obtained on each animal follows the tables relating to that experiment.

PROTOCOL. Dog 1, Fox terrier. Depancreatized November 1, 1925. Uneventful recovery during treatment with small quantities of insulin and moderate diet. Placed on a known diet November 17. Weight December 1, 5.57 kgm. On this date and thereafter till December 6 the dog received water but no food or insulin. The urine was collected quantitatively at 10:20 a.m., except on December 5 when the dog was catheterized at 9:20 a.m. and then placed in the respiratory cabinet. During the first half-hour she was restless and observations were not commenced until 10:30 a.m. after which they were continued till 12:30 p.m. when she was given 20 grams of dihydroxyacetone by stomach tube. The observations recommenced at 1:10 p.m. and were continued for four hours. She was catheterized at 11:05 a.m., December 6, when the experiment terminated. The results are shown in tables 1 and 2.

Comment. In table 1 the urinalyses show that on the two days preceding the administration of the dihydroxyacetone the glucose excretion was almost stationary, with the nitrogen excretion rising somewhat. The acetone bodies were increasing and were approximately two and one-half times as great in amount on the second day. During the day on which dihydroxyacetone was administered the excretion of nitrogen and glucose was increased and a small amount of dihydroxyacetone appeared in the urine immediately after the administration of the triose. This was not due to vomiting and the night specimens are always free from it. The excess

sugar excretion when 20 grams of triose was given amounted to 17.8 grams in twenty-five and one-half hours, using for its determination the D:N ratio of the second control day, 2.8. (Since one gram more nitrogen was excreted on this day than on the

TABLE 1

Dog 1. Urine examination

TIME	TOTAL GLUCOSE EXCRETION	TOTAL NITROGEN EXCRETION	D/N RATIO	TOTAL ACETONE BODIES	REMARKS
	grams	*grams*		*mgm.*	
Dec. 3–4	13.1	3.72	3.5	139	Depancreatized November 1
Dec. 4–5	12.7	4.5	2.8	741	
Dec. 5, 9:30 a.m.–5:50 p.m.	20.75	1.86		347	12:30 p.m. given 20 grams dihydroxyacetone. Urine contained 0.8 gram or less. Vomitus 30 cc. mixed with urine
Dec. 5, 5:50 p.m.–Dec. 6, 11:05 a.m.	12.5	3.68		1,450	Urine contains no dihydroxyacetone
Dec. 5, 9:30 a.m.–Dec. 6, 11:05 a.m. Total 25½ hr.	33.23	5.55		1,896	17.8 grams extra glucose excreted. Ketone bodies increased

TABLE 2

Dog 1. Weight 4.46 kgm. Respiratory quotients

TIME	O_2 CORRECTED	CO_2 PRODUCED	R.Q.	REMARKS
	cc.	*cc.*		
9:30–10:30				Fifth fasting day
10:30–11:30	3,675	2,489	0.677	
11:30–12:30	3,430	2,402	0.700	
12:30– 1:10				Control period; given 20 grams dihydroxyacetone at 12:30 p.m.
1:10– 2:10	4,485	2,973	0.663	Vomited small amount at 1:28; dihydroxyacetone very small in amount. 4:00 urinated
2:10– 3:10	4,260	2,754	0.646	
3:10– 4:10	3,542	2,428	0.686	
4:10– 5:10	3,754	2,494	0.664	

previous day it may be that a part of this is due to increased protein destruction. On the other hand it is our experience that the D:N ratio of fasting depancreatized dogs usually falls with continued fasting, and in that case less of the total sugar ex-

cretion would be attributable to protein destruction. In any case it is seen that the "extra glucose" approximates the dihydroxyacetone fed.) There were also increases in nitrogen and in acetone bodies but the extent of these was no greater than between the first and second control days.

The respiratory quotient is of particular interest. The animal behaved very well while in the cabinet, little movement being registered on the recorder. The control

TABLE 3

Dog 2. Male, black and tan. Urine examination

TIME	TOTAL GLUCOSE EXCRETION	TOTAL NITROGEN EXCRETION	D/N RATIO	REMARKS
	grams	*grams*		
Nov. 27–29	50.5	8.25	6.1	Water *ad lib.*
Nov. 29–30	14.4	3.83	3.75	
Nov. 30–Dec. 1	15.1	4.12	3.68	
Dec. 1, 10:55–12:00 noon	1.32	0.33	3.27	20 grams dihydroxyacetone at 12:00
12:00–1.17	7.35	0.20		25½-hour period
1.17–12:30 p.m. Dec. 2	23.2	3.92		
Total, Dec. 1–2	31.87	3.95		Extra glucose excreted 17.3 grams

TABLE 4

Dog 2. Male, black and tan. Blood examination

TIME COLLECTED	TOTAL BLOOD SUGAR	DIHYDROXY-ACETONE	TRUE BLOOD SUGAR*	REMARKS
	per cent	*per cent*	*per cent*	
Dec. 1				
12:00	0.270	0	0.270	20 grams dihydroxyacetone at 12:05
12:28	0.416	0.137	0.308	
12:55	0.440	0.131	0.337	
1:28	0.526	0.114	0.436	
1:58	0.456	0.035	0.429	
2:25	0.436	0.010	0.428	

* This represents the difference between the total reducing power of the blood filtrate (total blood sugar) and the reducing power of the dihydroxyacetone.

respiratory quotients are at the so-called "fat" level, while after administration of the triose there is, if anything, a slight fall in this value as is commonly observed after glucose administration. At the same time the consumption of oxygen increased indicating probably a specific dynamic effect of the dihydroxyacetone. In this we can observe the reason for a failure of antiketogenic action of the triose.

PROTOCOL. Dog 2, male, black and tan. Depancreatized November 12, 1925. Recovery uneventful. Allowed water but deprived of food and insulin November 26. Urine was collected as follows: November 27, 10:30 a.m., November 29, 1:30 p.m.,

November 30, 11:30 a.m., and December 1, 10:55 a.m. On December 1 the weight was 5.8 kgm.; at 12:00 noon urine was collected and a specimen of blood removed from the saphenous vein after which the dog was given 20 grams of dihydroxyacetone. Further samples of blood were taken at 12:28, 12:55, 1:28, 1:58 and 2:25 p.m. At 1:17 the dog vomited; vomitus 25 to 30 cc. It also urinated a small amount. Urine was finally collected at 12:30 p.m. on December 2 when the experiment was terminated with the animal in good condition. The results are shown in tables 3 and 4.

Comment. This experiment was undertaken principally to investigate the behavior of the blood sugar after administration of dihydroxyacetone. The urinalyses are of interest, however, in confirming the results obtained on the preceding animal. The nitrogen excretion was approximately the same throughout the test period except for the first of the control days when it was higher. The D:N ratio was approximately 3.7 on the two control days, and it fell to 3.3 on the day dihydroxyacetone was given. Calculating in the usual way, and allowing for material lost in the vomitus, 20 grams of dihydroxyacetone given to the animal reappeared as glucose in the urine.

TABLE 5

Dog 3. Depancreatized. Urine examination

TIME	TOTAL GLUCOSE EXCRETION	TOTAL NITROGEN EXCRETION	D/N RATIO	TOTAL ACETONE BODIES	REMARKS
	grams	*grams*		*mgm.*	
Dec. 3–4	6.38	2.77	2.31	28	Tenth day after pancreatectomy and third day of starvation
Dec. 4–5	6.0	2.93	2.05	245	
Dec. 5, 11:05–2:17	0.76	0.33	2.25	58	
Dec. 5, 2:17–5:23	13.5	2.5		19	20 grams dihydroxyacetone at 2:32. Dihydroxyacetone in urine less than 0.1 gram
Dec. 6, 11:10 a.m.	10.6	1.47		213	No dihydroxyacetone in urine
Dec. 5–6, totals	24.86	4.30		290	Extra glucose excreted 16 grams Ketone bodies unchanged

Regarding the blood findings (table 4) it can be seen that the total reducing power of the blood rose, following the administration of the triose. This increase is dependent on two factors: an actual increase in glucose, and the addition of the triose itself to the blood. The influence of the latter, as can be seen in the third column of the table, is marked at first, and then rapidly falls (in spite of the absorption of food which must still be going on) so that it practically disappeared in two hours and twenty minutes after its administration. The fall is not due to excretion of dihydroxyacetone in the urine, and coincident with it there was a decided rise in the true blood sugar, which is undoubtedly to be accounted for by the conversion of the triose into glucose. This finding corroborates those previously reported in observations on man and rabbits exhibiting insulin hypoglycemia (17). In addition it establishes the fact that insulin is not the agent responsible for the transformation of the triose into glucose, since in these cases no insulin was available.

PROTOCOL. Dog 3, female terrier. Depancreatized November 2[illegible]. Wound healed November 30. Given food and insulin till December 1; then observed in

fasting condition until December 6. The urines collected on the first three days were discarded. Animal catheterized at 10:30 a.m. December 3, at 11:05 a.m. December 4 and 5 and the urine was added to the twenty-four hour sample and the cage washings and made up to a definite volume. On December 5 the animal was catheterized at 11:05 a.m. and did not void thereafter. It was catheterized again at 2:17 p.m. and the specimen saved separately. From now on blood samples were taken at the periods indicated in table 6 and 20 grams dihydroxyacetone were given by stomach tube at 2:32 p.m. At 3:30 p.m. the animal vomited 5 cc. mucus which was collected separately, and found to contain 50 mgm. dihydroxyacetone. At 5:23 p.m. the animal was catheterized and the specimen saved separately. On December 6 the animal was catheterized at 11:10 a.m. after which the experiment was terminated by giving animal food and insulin. Cf. tables 5 and 6.

Comment. In tables 5 and 6 further corroborative evidence of the previous results is available. Ringer and Frankel (3), using phloridzinized dogs to which they administered dihydroxyacetone, and Sansum and Woodyatt (4) also using phlorid-

TABLE 6

Dog 3. Depancreatized. Blood examinations

TIME	TOTAL BLOOD SUGAR	DIHYDROXY-ACETONE	TRUE BLOOD SUGAR*	REMARKS
	per cent	*per cent*	*per cent*	
Dec. 5				
2:25	0.278	0	0.278	
2:32				20 grams dihydroxyacetone by
2:54	0.408	0.123	0.312	stomach tube
3:23	0.528	0.180	0.388	13.5 grams total sugar excreted
3:50	0.546	0.121	0.450	in urine during this period;
4:17	0.50	0.077	0.44	less than 0.1 gram is dihy-
4:44	0.484	0.040	0.46	droxyacetone
5:27	0.476	0.007	0.47	

* This represents the difference between the total reducing power of the blood filtrate (total blood sugar) and the reducing power of the dihydroxyacetone.

zinized animals to which they administered glucose by mouth, found "extra glucose" in the urine amounting to 60–100 per cent and 52–75 per cent respectively of the dihydroxyacetone or glucose administered. Our results on depancreatized animals show a high and in many cases a quantitative excretion of "extra glucose," indicating that all the dihydroxyacetone was converted into glucose. It may be remarked here that the prevailing custom (to which we have conformed) of calculating the sugar derived from protein in the test day from the D:N ratio of the previous day or from an interpolated value calculated from the D:N ratios of the days preceding and succeeding the test day, and the nitrogen excretion of the test day is open to several objections. The most important of these is the fact that the increased diuresis induced by the "extra glucose" often carries out with it "extra" nitrogen from the partially dehydrated tissues, which was not derived from protein metabolized on the test day. This often *results* in the excretion of more than a gram of extra nitrogen and consequently from 2 to 4 grams more sugar are credited to protein than is actually derived from it on the test day. Correction for this factor in most cases brings the true extra glucose even closer to the theoretical value.

PROTOCOL. Dog 4, female pug. Depancreatized December 1, 1925. Wound completely healed. Fed and given insulin on the morning of December 21 and none thereafter. Urines collected at 10:10 o'clock except where otherwise noted in table 7. The experiment was terminated on December 27 when the dog was in good condition.

Comment. In this animal the excretion of "extra glucose" after administration of dihydroxyacetone is practically a quantitative one. Attention may be called to the fact that in this series, as in others, there is no fixed level for the D:N ratio in completely depancreatized, fasting dogs. The present analyses were done in duplicate and, if necessary, they were repeated until satisfactory agreement was obtained. The animals thus examined all showed a respiratory quotient at the "fat" level and were subsequently carefully autopsied without a trace of pancreatic tissue being found.

TABLE 7

Dog 4. Female pug

TIME	TOTAL GLUCOSE EXCRETION	TOTAL NITROGEN EXCRETION	D/N RATIO	PHOSPHORUS	REMARKS
	grams	*grams*		*gram*	
Dec. 23–24	12.0	4.65	2.58	0.305	Third day of fasting
Dec. 24–25	13.6	4.96	2.75	0.252	
Dec. 25–26					Portion of specimen lost
Dec. 26, 10:20 a.m.–2:23 p.m.	1.78	0.71	1.78		
2:23–5:50	7.83	0.86			Dihydroxyacetone, 20 grams, given at 2:30 p.m.
5:50–Dec. 27, 10:40 a.m.	23.8	4.28			
Dec. 26–27, totals	33.41	5.85	0.0	0.465	20.2 grams "extra glucose" excreted. D:N ratio of 2.25 assumed
Dec. 27–28	12.8	6.5	1.97	0.42	

PROTOCOL. Dog 5, female Irish terrier. Weight 6.4 kgm. Depancreatized several months before; wound healed. Given food and insulin on the morning of December 21, 1925, and none thereafter. Urine collections were made at 10:00 a.m. unless otherwise stated in table 8. On December 26 the animal was placed in respiration cabinet at 9:45 a.m. Twenty grams dihydroxyacetone were given at 1:00 p.m.; animal vomited a considerable quantity. The experiment was terminated on December 27 with the animal in good condition. The results are given in tables 8 and 9.

Comment. In this animal the increased excretion of sugar following administration of dihydroxyacetone, when calculated in the usual way, exceeded that obtainable from the intake (part of which was rejected). The particular interest of the observation is that dihydroxyacetone caused no decrease in the excretion of the acetone bodies whereas that of phosphorus became distinctly greater.

The respiratory quotients in the preliminary period show satisfactory agreement and the dog behaved well until about 4:30 p.m. when she became rather restless and was removed from the cabinet at 4:40. A further hour's observation was obtained

from 6:00 to 7:00 p.m. The results of all four hour's observation, covering a period of six hours (including the control periods) following the ingestion of 20 grams of dihydroxyacetone, are identical with those of the preliminary period, the average

TABLE 8

Dog 5. Irish terrier. Urine examinations

TIME	TOTAL GLUCOSE EXCRETION	TOTAL NITROGEN EXCRETION	D/N RATIO	PHOSPHORUS	TOTAL ACETONE BODIES	REMARKS
	grams	*grams*		*gram*	*grams*	
Dec. 23–24	21.7	3.20	6.8	0.435	0.60	
Dec. 24–25	11.2	3.80	2.96	0.340	1.338	
Dec. 25–26	11.05	3.82	2.9	0.340	2.715	
Dec. 26, 9:40 a.m.–7:05 p.m.	18.2	2.0		0.148	0.930	In respiration calorimeter
						Given 20 grams dihydroxyacetone at 1:00 p.m.
7:05 p.m.–Dec. 27, 10:40 a.m.	13.0	3.4		0.392	1.805	25 hours
Dec. 26	7.9					Vomitus containing dihydroxyacetone
Dec. 26–27, totals	39.1	5.4		0.540	2.735	23.5 grams "extra glucose" excreted. Ketone bodies unchanged

TABLE 9

Dog 5. Irish terrier. Respiratory quotients

TIME	O_2 USED	CO_2 PRODUCED	R.Q.	REMARKS
	cc.	*cc.*		
10:30–11:30 a.m.	3,667	2,525	0.689	Day of fasting
11:30–12:30 p.m.	4,106	2,764	0.673	Quiet
				Given 20 grams dihydroxyacetone by stomach tube at 1:00 p.m.
1:40– 2:40 p.m.	4,486	3,074	0.685	Sleeping
2:40– 3:40 p.m.	4,103	2,764	0.674	Vomited small amount
3:40– 4:40 p.m.	4,231	2,922	0.691	Vomited
				Restless, removed from cabinet
6:00– 7:00 p.m.	4,418	2,988	0.675	Fairly quiet

respiratory quotient in each being 0.681. There is evidence of a greater oxygen intake following the ingestion for which the restlessness of the animal and the vomiting may, in part at least, be responsible.

PROTOCOL. Dog 1. This observation was made on the same dog as reported in table 1. Food and insulin were last given on December 31 at 10:00 a.m. Urine was collected in the usual manner between 7:40 a.m., January 3 and 9:40 a.m., January 4, after which the animal was placed in the respiratory cabinet till 5:45 p.m., receiving 20 grams dihydroxyacetone at 1:00 p.m. The urine (contaminated by some vomitus) was also collected during this period. Urine was subsequently collected between 5:50 p.m. on January 4 and 7:50 a.m. on January 5. The results are shown in tables 10 and 11.

TABLE 10

Dog 1. Urine examinations

TIME	TOTAL GLUCOSE EXCRETED	TOTAL NITROGEN EXCRETED	D/N	REMARKS
	grams	*grams*		
Jan. 3, 7:40 a.m.–Jan. 4, 9:40 a.m.	16.8	5.91	2.8	Fourth day of fasting. 26-hour period
Jan. 4, 9:40 a.m.–5:00 p.m.	19.5	0.95		20 grams dihydroxyacetone given at 1:00 p.m. 5 grams dihydroxyacetone in vomitus
Jan. 4, 5:00 p.m.–Jan. 5, 7:30 a.m.	11.9	2.86		22-hour period
Jan. 4, totals	31.4	3.81		20.8 grams "extra glucose" excreted

TABLE 11

Dog 1. Respiratory quotients

TIME	O_2 CORRECTED	CO_2 VOL.	R.Q.	REMARKS
	cc.	*cc*		
Jan. 4				
9:40–10:40				4th fast day. Control period
10:40–11:40	3,586	2,586	0.721	
11:40–12:40	3,759	2,677	0.712	
12:40–1:45				Control period. 20 grams dihydroxyacetone given at 1:00 p.m.
1:45–2:45	4,993	3,517	0.704	Vomited small amount at 1:50 p.m.
2:45–3:45				Leak discovered in 2nd circuit
3:45–4:45	4,035	2,738	0.679	On circuit 1

Comment. In this experiment it was proposed to make repeated observations in the respiratory chamber as was done in the following experiment. The develop-

ment of a leak in the second circuit made it impossible to carry this out and the remainder of the experiment was abandoned. The results, as shown in tables 10 and 11, are in agreement with the previous findings, practically all of the administered dihydroxyacetone being accounted for by the extra sugar excreted.

PROTOCOL. Dog 6, a fat young female fox terrier, had been depancreatized six months previously. The wound healed completely and the dog was fed liberally on meat, pancreas and sugar with appropriate doses of insulin in order to make her fat. She was last fed on September 8. Owing to the severe vomiting induced by withdrawal of the insulin, which always occurs in fat animals, four units of insulin had to be given on the mornings of September 10 and 11. This controlled the vomiting

TABLE 12

Depancreatized dog 6

TIME	TOTAL SUGAR EXCRETION	TOTAL NITROGEN EXCRETION	PHOSPHORUS PER DAY	TOTAL ACETONE BODIES	REMARKS
	grams	*grams*	*gram*	*grams*	
Sept. 12, 10:35 a.m.–Sept. 13, 9:10 a.m.	12.9	2.85	0.93	1.836	Fourth day of fasting. No insulin given. Insulin, 4 units, on previous two days
Sept. 13–14	23.0	4.95	0.60	3.696	Insulin, 2 units, at 5:00 p.m.
Sept. 14–15	20.1	4.21	0.545	2.010	Dihydroxyacetone in vomitus and urine, 5 grams. Insulin, 4 units, at 5:00 p.m.
Sept. 15–16	19.2	3.06	0.51	2.220	Vomitus and urine in respiratory cabinet contains dihydroxyacetone 3.7 grams
Sept. 16–17	25.95	4.53	0.41	2.371	No insulin. Dihydroxyacetone vomited and in urine, 3.7 grams
Sept. 17–18	27.0	4.4	0.50	2.258	No vomiting. No insulin

but it recurred on September 13 when two units of insulin were given at 5:00 p.m., after which the vomiting ceased, although it recurred on the 14th when four units of insulin again had to be administered at 5:00 p.m. Thereafter the vomiting became much less marked though a portion of the dihydroxyacetone was vomited on September 15 and 16 but not on September 17. The animal was placed in the respiration apparatus on September 14, 15, 16 and 17. During each of these days the twenty-four-hour urine was collected in the usual manner and analysed for phosphorus and acetone bodies, as well as sugar and nitrogen. The results are shown in tables 12 and 13.

Comment. This animal was made fat for other reasons than the present experiment. Owing to the development of dehydration, severe vomiting and acidosis which we have found always occurs in such animals it was necessary on several

occasions to inject small doses of insulin, as noted in the protocol. The D:N ratios are, therefore, of no significance, while the sugar excretion, except for the two last days of the test, is also affected by the insulin administered. On these days the

TABLE 13

Dog 6. Respiratory quotients

TIME	O_2 CORRECTED	CO_2 CORRECTED	R.Q.	REMARKS
	cc.	*cc.*		
Sept. 14				Fifth day of fasting. Weight 5340 grams
11:10–12:10	4,388	2,988	0.681	Quiet
12:10–1:10	4,173	3,011	0.721	19 grams dihydroxyacetone by stomach tube. Vomited part
1:15				
1:40				
1:45–2:45	4,550	3,120	0.685	Quiet. Vomited, 2:25
2:45–3:45	4,050	2,825	0.697	Quiet
Sept. 15				Weight 5142 grams
10:25–11:25	3,853	2,652	0.688	Moving about
11:25–12:25	4,010	2,853	0.711	Restless
12:30				20 grams dihydroxyacetone by stomach tube
1:10–2:10	3,593	2,408	0.670	Quiet
2:10–3:10	3,534	2,418	0.684	Quiet
3:10–4:10	4,014	2,825	0.704	Moving
Sept. 16				Weight 4990 grams
10:10–11:10	3,920	2,688	0.686	Moving about
11:10–12:10	3,943	2,825	0.716	Moving
12:30				20 grams dihydroxyacetone by stomach tube
1:15–2:15	4,194	2,957	0.705	Quiet
2:15–3:15	3,887	2,586	0.665	Quiet
3:15–4:15	4,195	2,968	0.707	
Sept. 17				Weight 4848 grams
10:10–11:10	3,540	2,179	0.615	Moving about
11:10–12:10	4,002	2,886	0.721	Restless
				Excitable
12:15				20 grams dihydroxyacetone by stomach tube
12:50–1:50	3,927	2,764	0.704	Moving considerably
2:06–3:06	4,286	3,029	0.707	Restless
				Quieter
3:06–4:06	4,173	2,942	0.705	Moving about

sugar excreted after administration of dihydroxyacetone is what would be expected from the results of the tests on other animals. The extent of the acidosis may be gauged from the ketone body excretion and is seen to have been practically uninfluenced by the administration of dihydroxyacetone.

Respiratory quotients before and after the administration of dihydroxyacetone were obtained on this animal on four successive days and the results are consistent in showing no rise in respiratory quotient. Increased consumption of oxygen followed the injection of dihydroxyacetone in the observations of the 14th and 17th, and to a lesser degree on the 16th, but in this regard the results are less striking than in the previous observations. Whether this less conspicuous specific dynamic effect is dependent on the fat condition of the animal we are not prepared to state. It is not related to the fact that some insulin had to be given since it occurred in the observation of the 17th, when this was not the case. So far as we know this is the longest series of respiratory experiments on a depancreatized dog and it is of added interest since some insulin had to be given this animal making the condition comparable with that of a diabetic patient.

SUMMARY AND DISCUSSION. In the preceding group of experiments there is supplied evidence that the triose, dihydroxyacetone, is quantitively excreted as glucose when administered to depancreatized dogs deprived of insulin and that no antiketogenic action of the triose can occur. A study of the concentrations of glucose and dihydroxyacetone in the blood after administration of the latter substance by mouth confirms the conclusion that it is absorbed into the blood stream as such, and gradually disappears from the blood without being excreted in the urine. Coincident with its appearance in the blood the blood sugar rises, and even although the animal is already hyperglycemic the true blood sugar also rises *pari passu* with the disappearance of dihydroxyacetone. It seems reasonable to conclude that since there is no other known source for this additional glucose in the blood it must arise from the disappearing triose. That the reducing substance which is found in the blood is actually glucose is shown by the following: It cures insulin hypoglycemia, which Herring, Irvine and Macleod have shown only substances convertible into glucose or mannose can do; it produces glycogen in the liver as Mostkowski has shown (17). It cannot be levulose since it fails to react with the phosphate molybdate reagent. It is excreted in the urine as a dextro rotatory fermentable sugar yielding an osazone identical with glucose. The present experiments also confirm the observations previously made on rabbits (18) that glucose is formed from the triose in excess of the amount needed to cure hypoglycemia. As insulin is absent in these experiments it is also clear that the hormone is not the agent responsible for causing conversion of the triose into glucose.

The behaviour of the respiratory quotient after administration of the triose is interesting in that it completely fails to rise from the usual levels obtaining in depancreatized dogs deprived of insulin. These results are identical with those obtained with glucose itself and the calculated respiratory quotient of each is the same: namely, unity. There is no possibility that the period of observation is too short to demonstrate increased combustion since the tables, 4 and 6, show that the substance disappears

from the blood in about two hours. Moreover respiratory quotient observations on successive days yield no support for this hypothesis (table 13). Under the conditions of the experiment there is a requirement of 16 to 20 calories per hour together with a need for utilizable carbohydrate for its protein sparing and antiketogenic effects. Although no evidence of any combustion of the triose could be obtained, it is significant that it has a specific dynamic effect as evidenced by the increased O_2 consumption which usually followed within an hour or so of its ingestion. Since the action of insulin probably takes place at the glucose stage, intermediary catabolites of glucose should not need insulin in their combustion. The excretion of the triose quantitatively as glucose is only evidence that, like other sugar formers, such as protein and various amino acids, it is metabolized through glucose and is not evidence that it is an intermediary stage in glucose catabolism.

From the clinical standpoint the obvious interpretation of these results must be that the triose cannot be metabolized more easily than glucose since it is actually metabolized through glucose and that it has no specific antiketogenic effect when tested on the only suitable subjects for deciding this question with any degree of finality.

CONCLUSIONS

Experiments with the triose, dihydroxyacetone, on depancreatized animals have shown that it is not metabolized in the absence of insulin but forms glucose and is excreted quantitatively as such. It raises the blood sugar of these animals and its conversion to glucose requires no insulin. Though its respiratory quotient is unity it does not raise that of the depancreatized dog when administered in adequate amounts without insulin. It is not antiketogenic in these animals. There is no evidence that it is an intermediary compound in the catabolism of glucose.

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THE EFFECTS OF DIETARY DEFICIENCIES ON THE GROWTH OF CERTAIN BODY SYSTEMS AND ORGANS[1]

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It has often been pointed out that growth involves two factors, the capacity for growth and the nutritive material necessary for the exercise of this capacity. Rubner (1908) suggested that maintenance needs of young, growing animals are satisfied before growth needs. The implication that, by adjusting the diet, maintenance without growth could be demonstrated, has stimulated extensive experimentation in the past. Waters (1908), Aron (1911) and Jackson (1915) using as experimental animals steers, dogs and rats respectively, have demonstrated that when the body weight is held constant for a considerable time, certain of the body systems and organs show a persistent growth at a retarded rate. These results were brought about by simple underfeeding of a presumably adequate ration—a quantitative deficiency. The present investigation involves a comparison of animals stunted by both quantitatively and qualitatively inadequate diets with normal animals of the same body weight. The dietary deficiencies dealt with may be described as *1*, calorie deficiency; *2*, protein deficiencies (diets containing a small percentage of protein, and diets containing protein with an unsatisfactory amino-acid make-up), and *3*, mineral deficiency. The data presented are concerned chiefly with the skeleton, brain, heart, liver and testes. It is thought that the fundamental question as to whether restriction of growth brought about in different ways produces like or different effects on the growth of the body systems and organs can be answered by the limited data presented. Jackson (1924) has given an excellent survey of the previous experimental work in this field.

EXPERIMENTAL PART. *Plan.* Healthy, young, male, albino rats were given the experimental diets when they had reached a weight of 40 grams (between twenty-five and thirty days of age). A series of twenty-five rats was fed on each diet, as this was considered the smallest number from which

[1] The data in this paper are taken from a dissertation presented by Jet C. Winters in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Yale University, 1926.

significant, statistical results might be obtained. The experimental diet was continued for a period of forty days, the time interval within which a 40-gram rat normally triples its weight. The animals were weighed each day and the diet adjusted, if necessary (as described below), to keep the body weight as nearly constant as possible. Since exact maintenance of weight was impossible, an increase of not more than 10 grams in the forty days was allowed. At the end of this period the animals were killed, the body and tail lengths measured and the hearts, livers, kidneys and testes removed and weighed. Certain bones were prepared and kept for measurement and weight comparisons. In order to obtain data on the growth of the brain and skull, cranial measurements were made for part of the rats of each series and actual brain weights obtained for the others. The technique used in the dissection of animals, the weighing of the organs and the preparation and measurements of the bones was similar to that in vogue at Wistar Institute and described by Donaldson (1924).

It was originally planned to use Donaldson's figures on normal rats for comparative purposes, but during the course of the work it became evident that the animals that were being used for this experiment were not closely enough like those of Donaldson to permit the use of his figures. The difference was most apparent in the weights of the livers and kidneys, the livers being considerably lighter (average 35 per cent) and the kidneys somewhat heavier (average 15 per cent) than those of Donaldson's animals of the same body weight. In addition to these differences our rats seem to be somewhat shorter and to have shorter and lighter bones than those of Donaldson. They are also considerably heavier than his rats of the same age. The finding of these differences, obviously, made it necessary to obtain normal figures for rats bred in this laboratory and similar to those used in the investigation.

Preliminary tests had shown that animals on the experimental diets usually weighed about 45 grams at the end of the experimental period, and it was evident that the average weight of a group of twenty-five would, in each case, fall close to 45 grams. Normal figures were therefore obtained by dissecting a series of rats showing an average weight of 45 grams and a variation in weight similar to that of the experimental animals, these rats being taken from a number of different litters. Comparison was made between these average figures for normal rats and the average figures for the experimental rats. Individual differences in the weights of the various organs, even of animals of the same body weight, from the same source, and on the same diet are large. It is therefore necessary to take these differences into consideration before drawing conclusions in regard to the variations between normal and stunted animals. This is done by the calculation of the significance ratio, as explained later. Control rats fed on the basal diet and given exactly the same amounts of vitamin-containing

materials as the stunted animals tripled their weight in about thirty-five days.

Diets. Below will be found the percentage composition of all diets used and a description of the way in which the ratio was adjusted when this was necessary to keep the body weight of the animals constant. Each rat was given 100 mgm. of dried yeast or 40 mgm. of Harris Yeast Vitamin Concentrate daily. The animals on the low-calorie diet received 20 drops of cod liver oil daily, while the animals on the other diets received only 5 drops. It was planned to give 20 drops of cod liver oil uniformly in order to be on the safe side so far as the fat-soluble vitamins were concerned, but it was soon found that only animals on the low-calorie diet would take this amount. It is probable that even 5 drops are far in excess of the actual need at this age. Control rats grew at a satisfactory rate on a daily intake of 5 drops. The casein used was a special preparation poor in fat containing 9.28 per cent of moisture, 0.87 per cent of ether extract, 13.45 per cent of nitrogen and 2.8 per cent of ash. The Osborne and Mendel (1919) salt mixture was used.

Basal Diet for Control Rats

(Energy value = 5.2 Cal. per gram)

	per cent
Casein	18
Cornstarch	51
Lard	27
Salt mixture	4

Low Calorie Diets

(Energy value = 5.2 Cal. per gram)

Diet A	*per cent*	*Diet B*	*per cent*
Casein	35	Casein	45
Cornstarch	27	Cornstarch	13
Lard	30	Lard	32
Salt mixture	8	Salt mixture	10

The high percentage of protein and inorganic salts in these diets was selected for the following reason: Previous investigators, in experimenting with underfeeding, have used small amounts of a ration supposedly adequate from a qualitative standpoint. It is probable that the effects produced in this way are not due to calorie insufficiency alone, but also to inadequacy of some one of the indispensable special dietary factors such as protein, salts or vitamins, since, in restricting calories, these are restricted also. The exact nature of the deficiency is difficult to determine as it varies with the intake of each of the dietary factors as well as with the amount of each stored in the body and with the rapidity of use. In the present experiments in order to find whether an improved growth would

follow an increase in protein and minerals, while the calorie intake remained the same, a number of rats after being held at maintenance for some time by feeding reduced amounts of the basal diet, were given a food of such composition that it provided "normal" amounts of protein and salts, i.e., the amounts that they would have received if they had been getting the normal intake of calories. An increased rate of growth was obtained which was interpreted as meaning that the animals were suffering from a protein and possibly a mineral, as well as a calorie deficiency. For this reason an effort was made to adjust the percentage composition of our low-calorie diets so that the possibility of a protein or mineral deficiency would be avoided. The adjustment was made on the basis of the following data:

Food intake of 40 gram rat on basal diet—4 grams per day.

Four grams of basal diet provide 0.72 gram of protein and 0.16 gram of salt per day.

Two grams of diet A provide 0.71 gram of protein and 0.16 gram of salt.

One and one-half grams of diet B provide 0.675 gram of protein and 0.15 gram of salt.

It was determined experimentally that rats could be kept near the maintenance level by beginning with 2 or 2½ grams of the food mixture and gradually decreasing to 1½ grams or 1 gram. Diet A was used at the beginning of the stunting period when about 2 grams of food were required for maintenance of weight and diet B was used later in the experiment when the food requirement fell to 1½ grams. That the amount of food necessary for maintenance of body weight decreases as the experiment progresses was also observed by Jackson (1915) in his experiments with underfed young rats.

LOW PROTEIN DIETS

Diet C	*per cent*	*Diet D*	*per cent*	*Diet E*	*per cent*	*Diet F*	*per cent*
Casein	1	Casein	3	Casein	5	Casein	6.5
Cornstarch	62	Cornstarch	60	Cornstarch	60	Cornstarch	58.5
Lard	33	Lard	33	Lard	31	Lard	31.0
Salt mixture	4	Salt mixture	4	Salt mixture	4	Salt mixture	4.0

It was invariably necessary to begin with diet C in order to check quickly the growth of an animal by means of the low protein diet. When the animal began to lose weight on diet C it was given diet D. If loss of weight continued diet E was used. It was seldom necessary to use diet F, though it was sometimes necessary to go from diet E back to diet D.

GLIADIN DIET

(Energy value = 5.2 Cal. per gram)

	per cent
Gliadin	18
Cornstarch	51
Lard	27
Salt mixture	4

For the most part no difficulty was experienced in keeping the rats on the gliadin diet at a maintenance level. Occasionally some of the animals lost slightly in weight. They were brought back to the beginning weight of 40 grams by a small increase in the amount of dried yeast supplied. The gliadin used was made in the laboratory and contained 16.5 per cent nitrogen. Gliadin is deficient in the amino-acid lysine and has been shown by Osborne and Mendel (1914) to be incapable of supporting growth when used as the sole source of protein in an otherwise adequate diet.

LOW SALT DIETS

(Energy value = 5.4 Cal. per gram)

Diet G	*per cent*	*Diet H*	*per cent*
Purified casein[2]	18.0	Purified casein[2]	18
Cornstarch	54.9	Cornstarch	55
Lard	27.0	Lard	27
Salt mixture	0.1	Salt mixture	0

It proved to be somewhat difficult to keep the animals at constant weight on a low salt diet. Even with as little as 0.1 per cent of added salt (diet G) many of the rats gained too rapidly. A few of the rats on a diet with no added salt (diet H) gained more than 10 grams in the forty-day period. Animals weighing more than 50 grams were discarded. The calorie intake on this diet was slightly below that on the other food mixtures.

Effects of dietary deficiencies on the growth of the skeleton. There is considerable evidence that inanition due to underfeeding is accompanied by persistent skeletal growth. (Waters, 1908 and 1909; Moulton, Trowbridge and Haigh, 1921; Aron, 1911 and 1914; Jackson, 1915 and 1922; Stewart, 1916 and 1919; Judson, 1916; Fleischner, 1906; Variot, 1907; Barry, 1920.) The proofs of skeletal growth during stunting brought about by qualitative dietary deficiencies are not conclusive. In the present experiments comparable degrees of stunting (practically maintenance of weight) have been produced by both quantitative and qualitative dietary deficiencies. The evidence relating to skeletal growth consists of measurements of body and tail lengths before and after the stunting period, comparison of the length of the stunted animals with that of normal animals of the same body weight, measurements and weights of the leg bones, crania and mandibles and histological examination of the bones.

In order to find the exact increase in body length during the stunting

[2] The casein used for the low salt diets was further purified by repeated washings with isoelectric water (pH 4.65). It was finally washed with alcohol and ether and dried in the oven. After this treatment, analysis showed nitrogen 14.5 per cent and ash 1.3 per cent.

period ten rats of each series were lightly anesthetized at the beginning of the period and their body and tail lengths measured. Comparable measurements were again obtained at the end of the period. Animals on a low-calorie diet showed an average increase of 10 mm. (9 per cent) in body length; those on low protein 13 mm. (12 per cent); those on a lysine deficient (gliadin) diet 18 mm. (16 per cent); and those on a low salt diet 13 mm. (12 per cent). This would tend to show that the increase in body length is least on the low-calorie diet and greatest on the gliadin diet. The lesser increase on the low-calorie diet is, however, not borne out by the observations on the larger series of rats (twenty-five) in which comparison is made between the average body length of the stunted animals and that

TABLE 1

Comparisons of the body length of normal rats and stunted rats of the same body weight

	AVERAGE BODY LENGTH	PERCENTAGE INCREASE IN STUNTED RATS	SIGNIFICANCE RATIO* WHEN COMPARED WITH NORMAL—$\frac{D}{PEd}$
	mm.		
Normal	120 ± 0.23		
Low calorie	125 ± 0.34	4	12
Low protein	125 ± 0.36	4	12
Gliadin	129 ± 0.39	8	20
Low salt	124 ± 0.40	3	8.7

* The significance ratio is determined by dividing the difference between the two averages (for normal and stunted rats) by the probable error of the difference—$\frac{D}{PEd}$. $PEd = \sqrt{(PEa)^2 + (PEb)^2}$ PEa being the probable error of the first mean and PEb the probable error of the second mean. If the quotient obtained is less than 3 the difference is not considered significant (D. C. Jones, *A First Course in Statistics*, 2nd ed., p. 160).

of normal animals of the same body weight. In table 1 data on the body length of normal and stunted rats are given. These figures comprise the average body length, the percentage increase in body length of the stunted animals, and results obtained by calculation of the significance ratios.

It is seen that the animals on the low-calorie and low-protein diets show the same amount of skeletal growth during the stunting period. The animals on the incomplete protein (gliadin) diet show an increase in length during the stunting period that is almost twice that of the animals stunted on low calories and low protein, while the animals on the low salt diet increase only about two-thirds as much as the low calorie and low protein animals and less than half as much as the gliadin ones. Considering the increase in length as an index of skeletal growth, it may be

concluded that the present data show persistent skeletal growth as a result of the four different kinds of stunting involved, the amount of this growth being greatest on the diet containing protein poor in quality and least on the low salt regime. Jackson (1915) found an increase of 10 per cent in body length in rats stunted by underfeeding from three to ten weeks of age, while Waters (1908) and Aron (1911) state, as a result of their experiments with steers and dogs, that the skeleton continued to grow at almost the normal rate.

The increase in length may be expressed in terms of percentage of normal growth attained. Control rats were allowed to grow at a normal rate until they had tripled the beginning weight of 40 grams. The average time required for this was approximately the same as the experimental period (40 days). At the end of the time required for tripling the weight, the control animals had reached a length of 166 mm., representing a gain of 46 mm. Animals on the low calorie and low protein diets gained only 5 mm. during the time in which the normal animals gained 46 mm. From these data it may be calculated that the low-calorie and low-protein animals made 10.8 per cent of the normal gain. In the same way it may be estimated that the gliadin animals made 19.5 per cent and the low salt animals only 8.7 per cent of the normal gain. The increase in length on the diet in which gliadin was the sole source of protein is contradictory to the opinion of Osborne and Mendel (1911) that rats on a diet of this kind remain unchanged in size.

Attention should be called to a possible error in obtaining the body length of stunted animals. Jackson (1915) stated that Donaldson called his attention to the arching of the spinal column that occurs during inanition. He observed such arching in his own stunted rats. Aron (1914) presents an x-ray outline of a rat held at maintenance for thirty days which shows the backbone folded in near the neck instead of being extended in a straight line. This arching was observed in the present experiment only in the rats on the low-calorie diet. Jackson (1915) thinks that the greater part of this longitudinal curvature is eliminated by the slight tension exerted in order to straighten out the body when it is measured after death, but it is possible that a slight source of error is introduced in this way. The rats on the low-salt diets sometimes develop abnormal forms, the most usual abnormality being a malformation of the bones of the pelvis resulting in a hump which prevents the body from lying flat on the table for length measurement. This does not often occur, but unless this abnormality is recognized it may introduce considerable error.

It has often been demonstrated that a stunted animal is a relatively long-tailed animal, i.e., in relation to body length the tail is always longer than in the normal animal of the same body weight. The data presented in table 2 confirm this idea. The percentage increase in tail length was

greatest in those animals whose increase in body length was least (low salt), the increase on all diets except the low-salt being nearly the same.

Table 3 shows the average length of the leg bones of normal forty-five gram rats and of rats of similar weight stunted by deficient diets, together with the percentage gains made by the stunted rats. The variation in the length of the leg bones of the animals stunted by different methods is

TABLE 2

Comparison of the tail length of normal rats and stunted rats of the same body weight

	AVERAGE TAIL LENGTH	PERCENTAGE INCREASE IN STUNTED RATS	SIGNIFICANCE RATIO WHEN COMPARED WITH NORMAL—$\frac{D}{PEd}$
	mm.		
Normal	99 ± 1.19		
Low calorie	111 ± 0.58	13	9
Low protein	110 ± 1.15	13	7
Gliadin	112 ± 0.81	13	9
Low salt	117 ± 0.87	18	12

TABLE 3

Average length of leg bones of normal rats and of stunted rats of the same body weight

	NORMAL	LOW CALORIE	LOW PROTEIN	GLIADIN	LOW SALT
Humerus—mm	14.9	17.0	16.4	16.9	16.5
Increase		13%	10%	13%	10%
Ulna—mm	16.3	18.7	18.4	18.8	18.2
Increase		14%	13%	15%	12%
Radius—mm	12.5	14.6	14.8	14.8	14.5
Increase		17%	18%	18%	17%
Femur—mm	17.1	21.0	20.0	20.5	20.4
Increase		23%	18%	18%	18%
Tibia—mm	22.1	25.5	25.1	25.4	25.2
Increase		15%	13%	15%	13%

so small that it may be said to fall within the limits of experimental error. The bones of the stunted animals are, however, from 10 to 20 per cent longer than those of normal animals of the same body weight. This points to an increase in height, somewhat greater than the increase in length. The experiments of Waters (1908) also indicated somewhat more striking increase in height than in length.

The lengths of the leg bones of the rats that have been allowed to grow normally during a period practically equal to the stunting period (average of four rats) were as follows: humerus, 20.7 mm.; ulna, 23 mm.; radius, 18.2 mm.; femur, 26.6 mm.; tibia, 30.8 mm. By computing the normal gain for each of the leg bones and comparing with it the gains made on the deficient diets (these being practically the same for each diet) it will be found that the humerus, radius and ulna made 36 per cent of the normal gain while the femur and the tibia made 41 and 39 per cent respectively. Thus, while the animals made only 8.7 to 19.5 per cent of the normally expected gain in body length, the leg bones made 36 to 41 per cent.

The average weight of the various bones of the stunted rats (table 4) is greater than normal as would be expected because of increased length. In all but those rats stunted with low salt diets, this weight increase

TABLE 4

Average weight of leg bones of normal rats and of stunted rats of the same body weight

	NORMAL	LOW CALORIE	LOW PROTEIN	GLIADIN	LOW SALT
Humerus—gram	0.039	0.068	0.060	0.060	0.041
Increase		74%	54%	54%	5%
Ulna and radius—gram	0.031	0.050	0.047	0.044	0.032
Increase		61%	51%	42%	0%
Femur—gram	0.067	0.129	0.111	0.115	0.080
Increase		90%	65%	71%	20%
Tibia and fibula—gram	0.069	0.113	0.104	0.111	0.079
Increase		63%	50%	61%	14%

amounts to 50 or 75 per cent and it is possible that this large difference is accounted for not only by the increased length but also by a difference in the ash content due to the diet used, and to greater age. Judson (1916) in her experiments on white mice fed on different diets found that the ash content of mice growing normally on an artificial food mixture of milk powder, casein, starch, salts and butter fat (protein 30 per cent, fat 32 per cent, starch 32.5 per cent and ash 5.5 per cent) is uniformly higher at corresponding stages of growth than the ash content of mice fed on a ration of mixed grains, dog-biscuit and small amounts of milk. This indicates that the ash content varies with the diet, and, considering a larger ash content to mean a greater weight, may account in part for the large differences in weight found in the present investigation. Our normal animals were fed whole wheat and milk after weaning for the short time required to reach a weight of 45 grams.

It is generally recognized that a decrease in the water content and an increase in the ash content of the tissues is a phenomenon of normal growth. Jackson (1915) found that during inanition in growing rats there was a steady increase in the percentage of dry substance in the growing cartilaginous skeleton, analogous, but not equal to that which takes place normally. He cites this as showing that the skeleton tends to grow in a normal manner. It seems possible, then, that a difference in age of the bones of the stunted animals and those of the normal animals may help to account for the differences found. Usually increase in the ash content of a stunted animal is taken to indicate increased skeletal growth. Should not this age factor be considered in such experiments, i.e., is there not the possibility that the bones have not increased in length to the extent indicated by the increase in the ash content, but that part of the increased ash content is due to a greater percentage of ash in the bones? The weight of the bones of the rats on the low-salt diet shows no great increase over the

TABLE 5

Average crania measurements and length of mandibles of normal and stunted rats of the same body weight

	LENGTH	CRANIUM WIDTH	HEIGHT	MANDIBLE LENGTH
	mm.	*mm.*	*mm.*	*mm.*
Normal	33.1	14.1	9.7	21.7
Low calorie	34.8	14.3	9.8	23.4
Low protein	34.5	14.3	9.6	23.1
Gliadin	34.6	14.2	9.6	23.3
Low salt	34.4	14.5	9.4	23.0

weight of bones of normal animals, but is 30 to 40 per cent below the weight of the bones of the animals stunted in other ways. Since the increase in length of the leg bones of the animals on the low-salt diet is no less than that of animals on the other diets, it appears that the bones continue to grow even while becoming depleted of minerals. These conclusions should be confirmed by actual ash analyses.

Table 5, giving the average measurements of the crania and mandibles, shows a slight increase in the length of the crania of the stunted animals over the normal animals and an increase in the length of the mandibles. This increase, however, is not very marked (3 to 4 per cent) but indicates continued growth of this part of the skeleton as well as of the parts already referred to.

Histological examination of the bones of the animals on the low-calorie, low-protein and incomplete protein diets showed osteoporosis and cessation of growth. The bones of the animals on the low salt diet showed cessation of growth and some of the characteristics of rickets, such as a slightly

increased amount of osteoid. The cartilage was, however, very thin and completely calcified. A subchrondral zone was present, similar to that commonly seen in incipient rickets. From the condition of the bone it was thought that the blood of the animals might have contained an abnormally large amount of phosphorus and a small quantity of calcium.

Effects of dietary deficiencies on the growth of the brain. Conflicting opinions will be found in the literature on the effects of dietary deficiencies upon the growth of the brain. The data presented in the present paper consist of the actual brain weights of a series of ten animals on each of the experimental diets, obtained at the end of the experimental period, together with a like series of brain weights for normal animals of the same body weight. The average brain weight for the normal and experimental animals is so nearly the same (normal 1.436 grams, low calories 1.439 grams, low protein 1.42 grams, gliadin 1.45 grams and low salt 1.474 grams)

TABLE 6

Comparison of weight of livers of normal rats and stunted rats of same body weight

	AVERAGE WEIGHT OF LIVERS	PERCENTAGE INCREASE IN STUNTED RATS	SIGNIFICANCE RATIO WHEN COMPARED WITH NORMAL — $\frac{D}{PEd}$
	grams		
Normal	2.12 ± 0.04		
Low calorie	2.15 ± 0.04	—	0.5
Low protein	2.92 ± 0.04	38%	8.0
Gliadin	2.21 ± 0.04	—	1.5
Low salt	2.65 ± 0.05	25%	8.0

as to lead to the conclusion that there is no increase in brain weight during the stunting period, regardless of the manner in which the stunting is brought about. When we compare the cranial measurements of the experimental and stunted animals (table 5), however, we find somewhat contradictory data. Although the skull lengths of the animals on experimental diets are practically the same, there is a slight increase (about 4 per cent) over those of the normal animals. This we should expect to be accompanied by a slight increase in brain weight. Since the figures for skull length are consistent throughout and since there are more sources of error in the determination of brain weight than in the determination of skull length, it is thought that the data here presented indicate a very slight increase in brain weight during the stunting period. There is apparently no increase in the height or width of the skull. Jackson (1915) concluded from data on rats maintained at constant body weight from three to ten weeks, that simple underfeeding brought about very slight,

if any, increase in brain weight. The weights of both the crania and mandibles of the stunted animals used in the present study showed a decided increase over that of normal animals. The weight of the crania of the low calorie rats increased 43 per cent, that of the low protein animals 39 per cent, that of the gliadin rats 38 per cent and that of the low salt rats 5 per cent. The mandibles, taken in the same order as above, increased 65, 51, 57 and 15 per cent respectively. These increases in weight can hardly be accounted for by the slight increases in length and are probably to be explained in the same way as the increases in weight of the leg bones. The lesser increase in the weight of the crania and mandibles on the low salt diet again indicates a probable loss in mineral content of the bones. The weight of the crania of the rats on the low salt diet is about 33 per cent less than that of the crania of the rats stunted in other ways; the difference in weight of the leg bones is also 30 to 40 per cent.

Effects of dietary deficiencies on the growth of the liver. Only the increases in liver weight on the low salt and low protein diets are significant (table 6). The increase in the weight of the liver of rats on the low salt and low protein diets is the same, the higher percentage increase on the low protein diet being offset by the greater probable error of the mean so that $\frac{D}{PEd} = 8$ in both cases. The failure to obtain an increase in liver weight on a low calorie diet is contradictory to the experience of Jackson (1915) who observed an increase of 10 per cent in liver weight in rats held at maintenance from three to ten weeks. When the stunting was begun later and continued longer, however, Jackson found a decrease in liver weight. Since the animals used in the present experiment were somewhat older and much heavier for their age than those used by Jackson, the difference in results may be accounted for in this way. The increase in the weight of the liver on the low-protein diet contradicts the experience of Watson (1907) who, using the wild Norway rat as an experimental animal, obtained a decrease in the percentage weight of the liver on a low protein diet of bread and milk. Rosenheim (1891), however, found hepatic enlargement on a protein-poor ration of rice, fat and small amounts of meat fed to dogs. Attention is called to the fact that the increase in liver weight found on the low protein diet was not found on the gliadin food. This affords an illustration of the different effects of the two kinds of protein deficiency. Evidence in the literature as to the effect of low salt diets on the growth of the liver is very conflicting and is based entirely on rations in which the lack of salt was complicated by other deficiencies. Since the histological examination of the bones of animals on the low salt diet showed a condition similar to beginning rickets it may be of interest to point out that the livers of the low salt rats resemble those of Jackson's rachitic rats so far as growth increase is concerned. Jackson and Carleton

(1923) found an increase in weight of 21 per cent in the livers of rats on a rachitic diet (but before the development of rachitic symptoms) and an increase of 36 per cent in the weight of livers of rats with beginning rickets. Macroscopically no changes were observed in the livers of animals on the low protein and low salt diets, but the observed weight increase needs to be supplemented by histological examination and determination of total solids in order to find whether it involves a true tissue increase, an edema or the production of abnormal tissue.

The average increase in the weights of the livers of rats growing normally until they had tripled the initial weight of 40 grams was 2.95 grams. Rats fed a low protein diet for the same length of time gained only 0.8 gram in liver weight. The gain in liver weight in this case was 27 per cent of the normal gain. The low salt rats showed a gain of 0.53 gram or 17 per cent of the normal.

TABLE 7

Comparison of the weight of the kidneys of normal rats and stunted rats of the same body weight

	AVERAGE WEIGHT OF KIDNEYS	PERCENTAGE INCREASE IN STUNTED RATS	SIGNIFICANCE RATIO WHEN COMPARED WITH NORMAL $\frac{D}{PEd}$
	gram		
Normal	0.582 ± 0.008		
Low calorie	0.589 ± 0.009	—	0.6
Low protein	0.570 ± 0.007	—	1.0
Gliadin	0.647 ± 0.006	10	6.5
Low salt	0.904 ± 0.004	55	36.0

The effects of dietary deficiencies on the growth of the kidneys. As seen in table 7 an increase in kidney weight occurs on only two of the deficient diets—lysine deficient (gliadin) and low salt. Jackson (1915) found only a slight increase in kidney weight in rats maintained at the same body weight from three to ten weeks by underfeeding, and no apparent difference when the stunting was begun at a later period. The lack of observed increase in kidney weight on the low calorie diet in the present study practically agrees with his observation.

The increase in kidney weight as a result of the gliadin diet though small was consistent. Since it does not occur as a result of the low protein diet, we have here another possible difference in the effects of low-protein and lysine-deficient diets. Kidneys of rats allowed to grow normally while tripling their body weight gave an average weight of 1.09 grams, an increase of 0.508 gram. The kidneys of the gliadin rats increased only 0.065 gram or 13 per cent of the normal gain.

The kidneys of the low salt rats increased 0.322 gram or 63 per cent of the normal increase, an observation which is one of the most significant results of this investigation. So far as has been found, this consistent increase in kidney weight as the result of an uncomplicated low-salt diet has not before been reported. Jackson and Carleton (1923) observed the weight of the kidneys 42 to 48 per cent above normal in rats with experimental rickets and also reported that the kidneys were 33 per cent above normal in weight on the rachitic diet, but before the appearance of rachitic symptoms. Their diets consisted of patent wheat flour supplemented by casein or lactalbumin, various salt mixtures and variable amounts of yeast and spinach and are obviously deficient in the antirachitic vitamin. In the present experiment a similar condition, increase in kidney weight, has been produced as a result of an uncomplicated salt deficiency. We have here evidence of the production of similar bodily conditions by two entirely

TABLE 8

Comparison of the weight of the hearts of normal rats and stunted rats of the same body weight

	AVERAGE WEIGHT OF HEARTS	PERCENTAGE INCREASE IN STUNTED RATS	SIGNIFICANCE RATIO WHEN COMPARED WITH NORMAL—$\frac{D}{PEd}$
	gram		
Normal	0.220 ± 0.004		
Low calorie	0.213 ± 0.002	—	1.5
Low protein	0.258 ± 0.003	17	7.6
Gliadin	0.235 ± 0.003	—	3.0
Low salt	0.239 ± 0.003	—	3.8

different kinds of dietary deficiencies. This phenomenon has already been demonstrated by the experiments of Sherman and Pappenheimer (1921) and others in which rachitic conditions have been produced and prevented by a shift in the mineral content of the diet without reference to the vitamin factors.

Effects of dietary deficiencies on the growth of the heart. The only significant difference in the heart weight of the stunted animals is the increase of 17 per cent as the result of the low protein diet (table 8). This increase, though small, was consistent throughout the series. It indicates still another difference in the results of the low protein and galidin diets. The failure of the heart to increase on the low calorie diet is in accord with results of Jackson (1915) on rats held at maintenance from three to six weeks of age. The failure to obtain an increase in heart weight on a low salt diet is, however, not in accord with the results of Jackson and Carleton

(1923) on rachitic rats in which an increase of 8 per cent was obtained in rats with beginning rickets and an increase of 15 per cent in rats on a rachitic diet but before the appearance of rickets.

The increase in the weight of the heart in rats growing normally during a period equivalent to the stunting period for the inadequately fed rats was 0.245 gram. The heart weight of the low protein animals increased only 0.038 gram or 15 per cent of the normal increase.

Effects of dietary deficiencies on the growth of the testes. The literature dealing with the effects of inanition on the testes indicates that the latter continue to increase in weight if the period of inanition comes before puberty. According to Donaldson (1924) sexual maturity occurs in the rat at about two months of age. As the present experiments were begun when the animals were about twenty-five days old and continued for forty days, the stunting period began before sexual maturity. There are greater variations in the weights of the testes than of the other organs and

TABLE 9

Comparison of the weight of the testes of normal rats and stunted rats of the same body weight

	AVERAGE WEIGHT OF TESTES	PERCENTAGE INCREASE IN STUNTED RATS	SIGNIFICANCE RATIO WHEN COMPARED WITH NORMAL $\frac{D}{PEd}$
	gram		
Normal	0.342 ± 0.009		
Low calorie	0.442 ± 0.020	30	4.5
Low protein	0.387 ± 0.020	—	2.0
Gliadin	0.500 ± 0.020	40	11.0
Low salt	0.435 ± 0.025	27	3.7

the significance ratios show that only the increase of weight in the testes of rats on the gliadin diet is of much importance (table 9). It should be borne in mind, however, that none of the rations contained vitamin E. That this accessory food factor is important in the sexual development of the male rat has been shown by Mason (1925).

Here again we have a striking difference in the effect of a low percentage of protein and an incomplete protein on the growth of an organ. Jackson (1915) found an increase of 34 per cent in the testes of rats held at constant body weight by underfeeding from three to ten weeks of age, but this figure is based on only seven observations. In rats with beginning rickets, Jackson and Carleton (1923) found a decrease of 17.5 per cent, although on a rachitic diet before rachitic symptoms had appeared there was an increase of 40 per cent.

The increase in the weight of the testes of rats growing normally during

a period equivalent to the stunting period of the inadequately fed rats was 1.318 grams, while the testes of the gliadin rats increased only 0.158 gram or 12 per cent of the normal gain.

Histological examination of the testes of two rats on each of the different diets indicated that the ration low in salts produces a more severe degeneration than the other foods used, although the animals on the inadequate energy regime showed degeneration slightly earlier than the above mentioned rats. Only incipient degrees of degeneration were observed in the testes of animals on the diets either poor in protein or containing a protein poor in quality.

SUMMARY AND CONCLUSIONS

Four groups of twenty-five young, male albino rats were kept at stationary body weight for a period of forty days by diets representing four different types of deficiencies,—inadequate energy (low calorie diet), inadequate protein, insufficient lysine (gliadin diet) and inadequate salt intake. At the end of the stunting period, the rats were dissected and data concerning the skeletal growth, growth of the brain and of some of the other more important organs obtained. By comparing these data with similar ones for normal rats of the same body weight and with data obtained on rats allowed to grow normally during a like period of time it was found that the growth of the body systems and organs was affected differently by different inadequate diets.

Persistent skeletal growth occurred on all diets used. This was manifested by an increase in the body and tail length and an increase in the length of the leg bones and skull. The gain in body length amounted to 3 to 8 per cent of the estimated body length at the beginning of the experiment (body length of rats of same body weight as experimental rats), or to 8.7 to 19.5 per cent of the normal gain. The increase in body length was greatest on the gliadin diet and least on the low salt one.

The weight of the leg bones of rats stunted on low calorie, low protein and gliadin rations was 50 to 60 per cent above that of the bones of the normal animals of the same body weight. This is due partly to increase in length, as shown by the measurements of the bones, but may also be due to other factors. The weight of the leg bones of rats stunted on low salt diets was 30 to 40 per cent below that of animals stunted in other ways, although the increase in length was the same. This applies also to the weight of the crania and mandibles and shows that the bones continued to grow even while being depleted of minerals.

The persistent skeletal growth apparently did not take place at the expense of any of the organs studied, as in no case was a decrease observed in organ weight.

The data are thought to show the possibility of a slight increase in brain weight on all the diets; no variation was apparent.

The most significant change brought about in the organs was a marked and consistent increase in the kidney weight as a result of the food low in salts. This increase amounted to 55 per cent of the estimated weight at the beginning of the experiment and to 63 per cent of the normal gain.

Other significant changes were an increase in liver weight on the low salt and low protein diets, an increase in the testes on the gliadin ration and in the heart on the low protein food.

The effects of low protein and gliadin stunting differed in all the comparisons made. The animals on the gliadin diet increased in body and tail length to a greater extent than those on the low protein food. There was an increase in kidney and testes weights on the gliadin diet which was not paralleled on the low protein diet, and an increase in liver and heart weights on the low protein diet which was not observed with the gliadin diet.

The effects of the ration low in salts on organ weight were, in most respects, similar to those found by Jackson in rats with incipient rickets.

There was in no case during the stunting period an increase in any part of the body comparable with the normal increase during a like period of time.

Grateful acknowledgment is extended to Dr. H. H. Donaldson of Wistar Institute for training in the technique of dissection, to Doctor Park and Doctor Winternitz of the Yale Medical School and Doctor Mason of the Osborne Zoölogical Laboratory for the histological examinations.

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THE EFFECT OF UNILATERAL NEPHRECTOMY ON THE GROWTH OF THE WHITE RAT

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The large margin of safety in kidney tissue is an accepted fact. As much as two-thirds of the total renal system of adults may be damaged or removed without fatal results. Yet, with very young animals, Arataki (1926) reported that unilateral nephrectomy "at 20 days tends to retard the growth in body weight without notably affecting the growth in body length." The present experiment purposed to test this thesis; to determine whether, by unilateral nephrectomy performed on very young rats, it is possible to interfere with the growth of the animals either in body weight or body length.

The reason for the alleged interference with growth brought about by unilateral nephrectomy is not obvious. Certain organs, the pituitary among them, exert a very definite influence on growth. On the other hand life is still possible when only one of practically all the paired organs remains intact and when parts of several of the unpaired organs are removed (Meltzer, 1906). Whether or not growth in young animals is possible under these conditions has not thus far been demonstrated.

It has been repeatedly shown that growth is dependent to a large extent on the quality and quantity of the diet. There is an irreducible minimum concentration of certain essential constituents of a ration—certain amino acids, inorganic salts and vitamins—below which ideal growth is impossible. Likewise the law of the conservation of energy applies to the growing animal—the quantity of the food must be adequate.

If the intake of food exerts such an important influence on growth is it not possible that this function may equally well be conditioned by the facility or difficulty of removing waste products from the body?

Numerous investigators have fed intact normal animals on rations containing extreme amounts of protein. In general, growth on such diets has not been so rapid as that on a lower protein food. Osborne, Mendel, Park and Darrow (1923) state that "the animals on such diets were poorly or at best only moderately well nourished." Their experimental diet contained 76 per cent casein. Reader and Drummond (1925) obtained growth at a subnormal rate on high protein food (caseinogen, 90 per cent),

whereas Jackson and Riggs (1926) reported good growth on a ration containing 76 per cent protein (casein and egg albumin). These experimenters did not give data on body length; body weight and general appearance were taken as criteria of growth. Accordingly in the present investigation one group of rats after unilateral nephrectomy was given an adequate ration containing an unusually large proportion of protein—a combination of circumstances calculated to bring a severe functional strain on the remaining renal tissue.

EXPERIMENTAL PART. Male white rats were used. The first group comprised fifteen animals 27 days old from each of which the right kidney had been removed aseptically under ether anesthesia.[1] The second group contained 9 rats of the same age. With similar technic, an incision was made, the right kidney being merely withdrawn to the exterior and replaced intact. This group served as the control. A third group of 6 animals 31 days old also was nephrectomized as described above. The recovery from the operations was prompt. Post-operative wound infection was observed in but one case, and was cleared up by drainage and irrigation with 1 per cent chloramine—T solution.

TABLE 1

Composition of diets used

	DIET 1		DIET 2	
	Part of food	Part of total calories	Part of food	Part of total calories
	per cent	*per cent*	*per cent*	*per cent*
Casein*	22	17	85	78
Corn starch	47	36	0	
Lard	22	47	7	22
Cod liver oil	5		4	
Salts**	4		4	

* A commercial "washed" casein containing 12.8 per cent nitrogen, 4.2 per cent ash.

** Osborne, T. B. and L. B. Mendel. 1919. Journ. Biol. Chem., xxxvii, 557.

The rats were given food and water ad libitum. The animals in the first and second groups were fed diet 1 (see table 1), a ration which has been shown repeatedly to be adequate for growth. Those in the third group were given diet 2 (see table 1), containing three-fourths of its energy as protein.

Each rat was given 150 mgm. of yeast daily aside from the other food. This was increased to 300 mgm. at 94 days of age and to 450 mgm. at 122 days of age in the case of third group, at 154 days in the case of first and

[1] The authors are indebted to Dr. T. S. Moise for assistance in removing the kidneys.

TABLE 2

Growth in body weight and body length

RAT NUMBER	27 DAYS OLD				94 DAYS OLD				131 DAYS OLD				201 DAYS OLD			
	Weight	Length		Body weight / Body length	Weight	Length		Body weight / Body length	Weight	Length		Body weight / Body length	Weight	Length		Body weight / Body length
		Body	Tail			Body	Tail			Body	Tail			Body	Tail	
	gm.	*cm.*	*cm.*		*gm.*	*cm.*	*cm.*		*gm.*	*cm.*	*cm.*		*gm.*	*cm.*	*cm.*	
1	43	11.9	9.8	3.61	192	19.8	17.9	9.70	247	20.9	19.6	11.91	275	22.1	20.0	12.44
3	44	12.1	11.7	3.64	166	19.5	17.8	8.51	214	20.5	19.3	10.44	240	22.6	19.8	10.62
5	55	13.2	11.2	4.16	199	19.7	18.3	10.10	231	20.2	19.1	11.43	238	21.4	20.0	11.12
7	36	11.2	9.6	3.21	170	19.3	16.8	8.81	210	20.1	18.3	10.45	235	21.4	19.0	10.98
9	43	12.1	10.6	3.55	196	19.9	18.1	9.84	226	21.0	19.1	10.71	228	21.4	20.3	10.65
11	40	11.7	10.7	3.42	181	19.5	18.5	9.28	216	20.7	19.0	10.43	230	21.5	19.6	10.70
13	35	11.5	9.4	3.04	199	20.1	18.3	9.90	248	21.3	19.5	11.64	268	21.8	20.1	12.29
15	46	12.5	11.5	3.68	204	19.9	18.6	10.25	256	21.0	19.9	12.19	260	21.8	20.7	11.92
17	37	12.2	10.2	3.03	197	20.0	18.6	9.85	232	20.3	19.3	11.43	233	22.1	20.2	10.54
19	46	12.7	11.0	3.62	197	20.1	18.6	9.80	240	21.4	19.0	11.21	251	22.1	20.6	11.36
20	49	12.7	10.4	3.86	183	19.6	16.7	9.34	236	20.8	17.8	11.34	257	21.6	18.5	11.90
22	44	12.4	10.4	3.55	180	19.8	17.6	9.09	218	20.5	18.5	10.63	235	21.4	19.4	10.98
23	43	11.8	9.8	3.64	206	20.0	18.6	10.30	257	21.5	19.7	11.95	280	22.6	19.7	12.39
24	39	11.7	10.4	3.33	196	20.3	18.9	9.65	244	20.6	19.7	11.84	264	22.3	20.5	11.84
25	41	11.8	10.3	3.47	168	19.0	17.6	8.84	200	20.9	19.2	9.57	210	21.5	20.2	9.77
Ave.	43	12.1	10.5	3.52	189	19.8	17.9	9.54	232	20.8	19.1	11.20	247	21.8	19.9	11.30
2	43	12.0	10.8	3.58	194	20.2	18.0	9.60	227	20.8	19.8	10.91				
4	41	11.8	10.2	3.47	193	19.3	17.7	10.05	218	20.8	19.2	10.48				
6	42	11.6	10.5	3.62	210	20.2	18.6	10.40	246	20.8	19.2	11.82	260	21.9	20.8	11.87
8	41	12.3	10.8	3.33	182	19.8	17.0	9.19	206	20.3	18.5	10.15				
10	47	12.4	11.0	3.79	198	20.3	18.5	9.75	238	20.2	19.4	11.78	249	21.9	20.1	11.37
12	43	12.3	11.2	3.49	167	19.3	17.0	8.65	206	20.3	17.8	10.15				
14	38	11.8	10.6	3.22	178	19.8	17.6	8.99	208	20.7	18.6	10.05	220	21.1	19.2	10.43
18	42	12.2	10.9	3.44	187	19.7	17.7	9.49	225	20.7	19.1	10.87	258	21.8	19.9	11.83
21	43	11.6	10.9	3.71	173	19.4	18.1	8.91	210	20.5	18.6	10.24	238	21.3	19.3	11.17
Ave.	42	12.0	10.7	3.51	187	19.8	17.7	9.45	220	20.5	18.9	10.72	245	21.6	19.9	11.33
	31 DAYS															
27	54				181	18.3	17.2	9.89	203	19.5	18.3	10.41	220	20.5	19.4	10.73
28	55				161	20.3	17.8	7.93	164	18.9	17.7	8.68				
29	54				189	19.2	17.6	9.84	210	20.1	18.1	10.45	200	20.4	19.1	9.80
31	50				166	18.3	17.1	9.07	180	19.0	17.4	9.47	180	19.9	18.6	9.05
32	41				146	17.6	16.9	8.29	168	18.8	17.2	8.93	175	19.7	18.3	8.88
33	63				182	19.7	18.0	9.24	186	19.9	18.5	9.35	180	20.1	17.9	8.96
Ave.	53				171	18.9	17.4	9.04	185	19.4	17.9	9.55	191	20.1	18.7	9.48

second groups. (See T. B. Osborne and L. B. Mendel, Journ. Biol. Chem., 1922, liv, 739.)

The routine care of the animals was that described by Smith, Cowgill and Croll (1925). The rats were weighed twice a week until the last two months, when they were weighed once a week.

Typical growth curves for individuals of the three groups are shown in the chart below.

At the time of operation all but the third group were measured by means of adjustable callipers. As soon as the animal was etherized, it was laid on its back and gently extended. Body length measurements were taken from tip of nose to anus; tail measurements from anus to tip of tail. Twice later in the course of the experiment the entire number of experimental animals was anesthetized and measured again. The final measurements were made when the experiment was ended.

The figures for body weight corresponding to the body length measurements are given in table 2. Several of the animals became affected with a respiratory disease and the blanks at 201 days indicate that the rats had been killed.

In addition to body weight the ratio of body weight to body length has been used as an index of good growth. Donaldson (1924) has given data showing an average ratio of body weight to body length. As might be expected this ratio varies somewhat with each animal and with groups of animals kept under different conditions.

It has been found that as a result of stunting by underfeeding there is a tendency for the skeleton to grow though the body weight remains constant with the consequent diminution of value of the ratio $\frac{\text{body weight}}{\text{body length}}$. (See Jackson, 1924.) That other methods of stunting produce like results has been shown by Winters, Smith and Mendel (1927). In the light of these facts a significant decrease of this ratio from the normal in the experimental group in the present investigation can be taken to indicate an inhibition of growth. Table 3 contains the data showing the results of the comparisons together with the explanation of the method of calculation used.

The data in table 3 show that between the control and nephrectomized groups on diet 1 there is no significant difference in body weight either at 27 days, 94 days, 131 days or at 201 days of age. The ratios of body weight to body length of the nephrectomized animals on diet 1 are very close to those calculated for the control group. All of the rats were sleek, fat and contented. Hence it appears that the readjustment of function and structure incident to unilateral nephrectomy does not, per se, alter growth rate either in body weight or body length.

On the other hand, data on a small number of animals show that nephrectomized rats on a protein-rich ration do not grow as rapidly as the

controls. The variation in body weight of group 3 from the normal is shown to be significant when the animals are 131 days old and to increase with the age of the rat. Though the body weight of the high-protein group was somewhat lower than that of the controls at 94 days, the deviation of the ratio $\frac{\text{body weight}}{\text{body length}}$ from the normal was not significant at that time. At 131 days a real difference appeared. This increased at 201 days for the growth curve of the normal was still ascending whereas that of the high-protein group approximated a level. The body length

TABLE 3

Variation of groups from normal

GROUP	MEAN BODY WEIGHT	SIGN. DIFF. FROM CONTROL	MEAN RATIO $\frac{\text{BODY WEIGHT}}{\text{BODY LENGTH}}$	SIGN. DIFF. FROM CONTROL
	94 days			
	grams			
Diet 1:				
Control	187		9.44	
Nephrectomy	189	−	9.55	−
Diet 2:				
Nephrectomy	171	−	9.04	−
	131 days			
Diet 1:				
Control	220		10.77	
Nephrectomy	232	−	11.15	−
Diet 2:				
Nephrectomy	185	+	9.55	+
	201 days			
Diet 1:				
Control	245		11.34	
Nephrectomy	247	−	11.31	−
Diet 2:				
Nephrectomy	191	+	9.48	+

Method of Calculating to Determine Significant Difference:

$$\text{Standard Deviation} = \sqrt{\text{average of squares of deviations from mean}}$$

$$\text{Probable Error} = \frac{\text{Standard Deviation}}{\sqrt{\text{Number of cases}}} \times 0.6745$$

$$\text{Probable Error of Difference} = \sqrt{(\text{P. E. mean}_1)^2 + (\text{P. E. mean}_2)^2}$$

When $\text{Mean}_1 - \text{Mean}_2 = 3 \times$ probable error of the difference, the difference ($\text{Mean}_1 - \text{Mean}_2$) is significant.

of these animals was not so great as that of the normals, but corresponded more closely to the body length of a normal rat of the same weight. The animals in the high-protein series had somewhat coarser hair, were not nearly as fat and did not appear to feel so well after they were half grown. These results as well as those on intact animals recorded in the literature suggest that a disturbance in the normal equilibrium between ingested protein and excretion of nitrogenous metabolites may be reflected in the growth rate.

A comparison of the food consumed reveals no difference between the amount eaten by group 1 and that eaten by group 2, throughout the entire experiment. Owing to the scattering of the protein-rich food, accurate intake data were not obtained. Reader and Drummond (1925) reported definitely increased food consumption by intact rats fed high-protein diets.

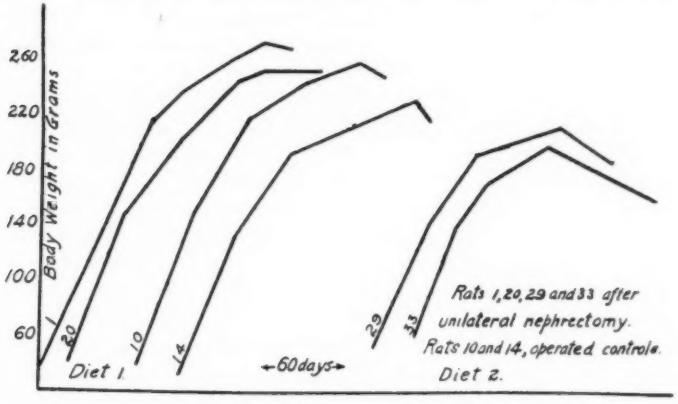

Chart showing typical curves of growth

Data reported elsewhere indicate that after unilateral nephrectomy adult rats on diet 2 consume less food calories than control animals on diet 1. (See Smith and Moise.[3])

The amount of yeast fed daily to the third group was increased from 300 mgm. to 450 mgm. at 122 days of age in order to insure sufficient vitamin B. This increase in yeast had no noticeable affect on the growth rate of the rats. The possibility of a vitamin deficiency as a cause of the stunting observed on the high-protein ration is thus eliminated. (See Reader and Drummond, 1925.)

Histological examination of the remaining kidney after unilateral nephrectomy showed that no damage had occurred in the kidney structure in either group 2 or 3.

[3] Smith, A. H. and T. S. Moise. 1927. Journ. Exp. Med., xlv, 263.

CONCLUSION

When the total renal tissue of young rats is reduced by unilateral nephrectomy the growth to adult size as measured by the increment in body weight and body length is not disturbed provided a ration containing a moderate concentration of protein is given. However, when similarly operated animals are fed a diet of extreme protein content, the early rapid growth ceases at a level definitely below that attained by the control rats in the same time interval.

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THE CIRCULATORY MINUTE VOLUMES OF HEALTHY YOUNG WOMEN IN RECLINING, SITTING AND STANDING POSITIONS

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No measurement in the field of circulatory phenomena is more important than that of the minute volume of the heart's output, yet well-established data are still meagre, obviously because this measurement on human beings is by no means simple. In particular, such data on young women are very few. The so-called healthy young women seen in college groups often show signs of circulatory inefficiency. Therefore it appeared desirable to make a series of careful observations on their circulatory minute volumes since a correlation might appear between such measurements and the success of the circulation in meeting daily needs.

The effect of posture rather than of exercise was selected as a place of attack because the latter is much better known (Boothby, 1915; Lindhard, 1915; Means and Newburgh, 1915; Collett and Liljestrand, 1924b; and others), and because,—and this was the determining factor,—the change from a reclining to an erect position seemed particularly hopeful for the study in question. It has been noted that quiet standing for a considerable time is frequently accompanied by signs of circulatory embarrassment or distress even in healthy individuals. The fainting of West Point cadets at inspection is one of many illustrations. It seemed highly probable, therefore, that this postural change might yield interesting results when studied from the standpoint of the minute output of the heart. It might be expected that the circulatory minute volume would rise when the standing position is assumed after a control period of reclining, since standing is more work and in general the circulatory minute volume has been found to run parallel to the needs of the body in exercise. There were, however, three suggestions that this might not be so. One of these is a brief study by Lindhard (1913b) of the effect of posture on the circulatory minute volume in a small group of subjects, where in some of the tests lower values were found for sitting and standing than for reclining, a change more pronounced in the three women than in the four men of the group. Field and Bock in a series of observations since published (1925)

had found in a larger group, nearly all men, that the decrease in the circulatory minute volume on prolonged quiet standing was conspicuous. Collett and Liljestrand (1924a) in one set of three experiments on Miss Collett found the volumes for reclining, sitting and standing to be 4.9, 4.0 and 3.6 litres per minute respectively. A suggestive finding, also, is that of Barcroft and Marshall (1923) that the minute volume of one subject (Barcroft) falls progressively as quiet sitting is prolonged after, presumably, the effort of preceding exercise might be over. It was therefore decided to make a systematic and careful study of the circulatory minute volumes of a group of healthy young women reclining, sitting and standing. This was done during the year 1924–1925 with the counsel and support of Dr. Cecil K. Drinker.

METHOD. The values obtained for circulatory minute volumes have varied with different observers and different methods. Clearly the measurements on human subjects must be indirect. The methods most used have been *a*, those employing as an essential feature the absorption of nitrogen or nitrous oxide by the blood in the lungs; *b*, those employing Fick's principle by which if the total oxygen consumption or carbon dioxide elimination per minute is known and also the comparative values of oxygen or carbon dioxide in arterial and venous blood, the amount of blood moving per minute can be calculated. The details of methods have varied. Valuable descriptions of the methods used and critical summaries of the results obtained may be found in Lindhard (1916), Haldane (1922), Barcroft and Marshall (1923), Henderson (1923), Means (1924), Barcroft (1925). It is conspicuous in the literature that the number of observations on women is much smaller than that on men.

The values obtained by the nitrous oxide method, even when the "correction" employed at first by Krogh and Lindhard is omitted as it is in the later calculations of those using this method (Boothby, 1915; Lundsgaard, 1916–a, b; Lindhard, 1922; Liljestrand and Stenstrom, 1925–a, b, c), seem to be lower than those obtained by various modifications of Fick's principle (Christiansen, Douglas and Haldane, 1914; Douglas and Haldane, 1922; Barcroft and Marshall, 1923; Burwell and Robinson, 1924; Field, Bock, Gildea and Lathrop, 1924; and others). But since by each method certain comparative relationships appear to hold and since after all the relationship of the minute volume at a particular time to the resting volume characteristic of the individual is usually of prime importance, the general discrepancies in results by the different methods may be of less significance than might be feared. The ethyl-iodid method of Henderson and Haggard (1925 a, b) seems to give results which are as large or larger than those obtained by using Fick's principle, but it is still too soon to appraise this method which presents both theoretical and experimental difficulties not easily overcome. A critical study which

calls attention to these difficulties is that of Moore, Hamilton and Kinsman (1926).[1]

The Field-Bock method practically as described by them (Field, Bock, Gildea and Lathrop, 1924) was chosen for this study and acknowledgment is made of their courtesy in sharing details of procedure. The essentials of that method of determining the circulatory minute volume during rest are:

1. The taking of a routine basal metabolism by the usual Benedict-Roth technic from which by the use of the average respiratory quotient of 0.81 or 0.82 the output of carbon dioxide per minute is derived.

2. The determination of the CO_2 of alveolar air which gives the CO_2 of arterial blood.

3. The determination of the CO_2 of a gas mixture which at the start is about 95 per cent oxygen and 5 per cent carbon dioxide and which is brought by a series of five or six short periods of rebreathing into CO_2 equilibrium with the mixed venous blood entering the lungs.

4. From 2 and 3 the difference in carbon dioxide content between arterial and venous blood is determined and Fick's principle can be applied. Dividing the output of CO_2 in cubic centimeters per minute by the change in cubic centimeters per litre of blood gives obviously the number of litres passing through the lungs per minute, and hence the heart's output.

The apparatus devised by Field and Bock makes it possible to secure arterial and "virtual venous" samples within a few seconds of each other and hence to approximate a true comparison of arterial and venous blood at a given time. The coöperation required of the subject is not greater than that of the usual basal metabolism test. After a series of determinations in the reclining position exactly the same procedure was followed either for a quiet sitting position or for quiet standing. Thus the effect of change of posture on the circulatory minute volume became apparent.

The subjects chosen were young women, some of them college students, some of them teachers, secretaries, graduate students and technicians.

[1] The number of authors using the method of Henderson and Haggard has increased since this article was prepared. The following papers include material of interest either from the point of view of technical improvement of the method or from the results obtained.

Codero, N. 1926. This Journal, lxxvii, 91.

Cullis, W. C., O. Rendel and E. Dahl. 1926. Journ. Physiol., lxii, 104. The authors find the circulatory minute volume in 12 women, studied in 57 experiments, under standard conditions, one to one and one-half hours after a light breakfast, semi-reclining position, to average 7.5 litres. This is somewhat higher than the determinations by other methods.

Henderson, Y. 1927. Journ. Physiol., lxii, 262.

Rosen, I. T. and H. L. White. 1926. This Journal, lxxviii, 168.

TABLE 1

*Data regarding subjects**

SUBJECT	AGE	HEIGHT		WEIGHT	SURFACE AREA	VITAL CAPACITY	REMARKS
		Standing	Sitting				
		cm.	*cm.*	*kgm.*	*sq. m.*	*l.*	
B.	19	177.7	92.2	68.0	1.83	3.77	Simmons 1926. Excellent posture. Healthy
H.	32	172.0	89.0	65.0	1.77	3.55	Research ass't. Hollow back. Possible low thyroid
J.	32	171.6	87.2	63.9	1.76	3.60	Simmons 1926. Posture fair
T.	28	170.5	87.4	59.2	1.69	3.73	Grad. student. Occasional dizzy attacks. Hollow back
P.	22	168.0	87.9	56.9	1.64	3.67	School Phys. Ed. Fine posture. Excellent condition
C.	25	167.4	89.2	62.0	1.70	4.60	Simmons 1926. Posture poor. Prominent abdomen
L.	22	166.8	85.4	57.8	1.65	3.12	Grad. Student and laby. ass't. Posture poor. Slumped
A.	21	166.7	87.5	54.0	1.59	3.50	Simmons 1925. Posture fair. Easily winded in sports
G.	34	165.9	85.6	55.5	1.64	3.88	Grad. student. Posture good
W.	31	165.5	86.0	54.7	1.60	3.96	Research, chemistry. Posture good. Tennis. Sturdy
V.	26	165.3	85.9	53.5	1.57	4.10	College instructor. Posture good. Healthy
U.	24	165.1	87.0	50.1	1.54	3.32	College instructor. Posture good. Not strong or athletic
X.	19	164.7	87.2	57.0	1.62	3.64	Mt. Holyoke 1927. Posture good. Sturdy. Tennis
O.	21	164.5	88.5	56.2	1.61	3.41	School Phys. Ed. Fine posture. Excellent condition
Q.	20	164.4	85.2	61.1	1.67	4.22	School Phys. Ed. Fine posture. Very athletic
K.	17	163.4	87.1	57.9	1.62	2.99	Simmons 1927. Posture fair. Not athletic
N.	20	163.3	85.2	50.8	1.53	3.09	School Phys. Ed. Fine posture. Losing weight
M.	33	160.7	85.8	58.6	1.61	3.50	Artist's model. Posture good. Muscularly soft
R.	19	160.5	83.7	55.2	1.57	3.30	Simmons 1927. Posture good. Cautioned about over-exertion
I.	22	158.6	83.0	53.6	1.54	2.80	Simmons 1926. Posture poor. Relaxed abdomen. Restless
S.	37	158.1	81.1	52.0	1.51	2.98	College instructor. Posture excellent. Radium, enl. thyroid, 1924
Y.	36	156.1	83.6	60.5	1.60	2.72	Secretary. Posture fair. Squarely built

TABLE 1—*Concluded*

SUBJECT	AGE	HEIGHT		WEIGHT	SURFACE AREA	VITAL CAPACITY	REMARKS
		Standing	Sitting				
		cm.	*cm.*	*kgm.*	*sq. m.*	*l.*	
F.	19	154.3	82.0	46.5	1.42	3.51	Simmons 1927. Greek. Thin. Relaxed abdomen
D.	26	154.2	81.8	65.0	1.63	2.58	Technician. Posture poor. Abdomen prominent. Squarely built
E.	22	148.8	78.1	45.0	1.36	2.16	Simmons 1925. Posture good. Not athletic. Small frame

* Arranged in order of height.

Four were students at the Boston School of Physical Education and one an artist's model accustomed to posing and also to being a subject for research in metabolism. All were in good health at the time of the experiments as far as known. They varied in age from 17 to 37, in height from 148.8 to 177.7 cm.; none were of unusual proportions. Data regarding them are given in table 1. Practically all had some sort of interest in the experiments, so the results may be considered to be free from lack of coöperation. There was usually a preliminary appointment when the subject was measured and when she found out what the procedure would be like. The program of an experimental morning follows in order to emphasize the precautions taken to procure dependable results in a field full of pitfalls.

The subject came to the laboratory as early as convenient, without breakfast, and reclined for 30 to 45 minutes. During this time readings were made of pulse, body temperature, room temperature and barometer. Three determinations of the basal metabolism were then made by the Benedict-Roth apparatus, with a rest period of about five minutes between each two tests. In the last half of the series a blower was used to move the air. This increased the comfort of breathing but while it lessened lung ventilation it had no influence upon total metabolism. A series of four determinations by the Field-Bock apparatus followed. This is so constructed that the subject with no change of mouthpiece may be connected with room air, with a tube for alveolar samples, or with a gas bag, which may contain any desired mixture of gases. For these experiments the bag contained an amount of gas somewhat below the subject's vital capacity, made up of about 95 per cent oxygen, to insure complete and uniform oxygen saturation of the blood during the tests, and 5 per cent carbon dioxide to prevent loss of carbon dioxide from the blood. Preliminary determinations showed the amount of gas which the subject was

likely to find convenient and after the first day her venous carbon dioxide was known so that the percentage of that gas could be approximated more closely in filling the bag. The subject's respiration was recorded by a pneumograph for about a minute before each test and during it to check the character of the breathing. Some subjects when they knew a test was about to be made tended to overbreathe and others tended to hold the breath. In some there was a peculiar little catch in the respiration before the deep expiration of an alveolar sample. Such points were watched for in preliminary trials and the experiment was not begun until the subject seemed to be at ease, able to breathe normally before the test and likely to do the test itself correctly. The possibility of altered respiration just before the test is the greatest danger in the method and the utmost care was taken to avoid it. Whole experiments were discarded and parts of others ruled out because the kymograph records showed the subjects had not breathed rightly before or during the tests. The same type of mouthpiece was used throughout all the experiments and breathing through it soon became unconscious.

The steadiness of the subject's state was further checked by her heart rate taken just before each test. In a few experiments continuous records of the pulse were made on the kymograph but the variations during reclining were so slight that this was not made a routine procedure.

When the subject was breathing evenly she was asked at the end of an ordinary expiration to give a forced expiration. By turning the cock at the proper instant this expired air was turned down the tube for alveolar samples. The position of deep expiration was held long enough for a cock to be turned to connect the subject with the gas bag, from which she then took a series of four or five deep breaths, ending with a deep expiration which left in the bag approximately as much gas as at the start. Two periods of such rebreathing were always done as a preliminary to each series of tests in order to bring the air in the bag into complete equilibrium with the venous blood before beginning the experiment proper. The alveolar sample was drawn from the tube the instant after the turning of the cock connected the subject with the bag, and the bag sample was drawn as soon as the rebreathing was finished. Both samples were taken into mercury sampling tubes previously made ready. Four pairs of samples were taken for each experiment at intervals of about three minutes and all samples were large enough to permit three analyses if necessary.

If a series of sitting tests was to be made after the reclining series, the subject then sat for a half-hour period in a comfortable chair of a design to favor a good sitting posture. A footstool was provided if desired. The aim was to establish a quiet sitting posture which could be easily maintained without restless movements or fatigue. At the end of the

half-hour allowed for complete adjustment, the whole series of metabolism and breathing tests was performed exactly as for the reclining position. A few subjects also did experiments in which a stool of comfortable height but with no support for the back was used instead of a chair.

On another day, each subject did a reclining and then a standing series of tests. It was found very early that tests in the standing position would prove more troublesome because of the intrinsic difficulty of standing still. Almost no one when standing stands *still*, a fact which in itself may show a protective reaction. The program of the experiments was, therefore, planned with short periods of standing of about eight to fourteen minutes in order to have the subject in a state which indeed was not indicative of her reaction on *long* standing, but which showed parallel results for several shorter periods. Both restlessness and fatigue were thus much decreased. After the reclining tests, the subject sat in a chair for about fifteen or twenty minutes. She then stood and was attached in a trial way to the metabolism apparatus. A brief period of breathing followed to familiarize her with the use of the apparatus in the standing position. After sitting for about five minutes the subject then stood while a metabolism test was taken. Three such tests were made, with a few minutes of sitting between each two periods. It was found that the records were less constant if the periods were prolonged so in the main the time covered by the test was six minutes in length instead of eight as in the reclining position.

After a sitting period the Field-Bock test followed—first a preliminary standing period of adjustment with two rebreathings from the bag, then after a rest of about five minutes (sitting position) the subject stood while four pairs of determinations were made. It was found frequently during the standing series that the subject's heart rate rose progressively. The control pneumograph record almost always showed less regular breathing than in reclining even though the subject appeared to be standing easily. The time required by the Field-Bock test took about six minutes longer than a single metabolism determination.

Following the series of reclining and either sitting or standing determinations, a Schneider test was made (Schneider 1923).

In the first preliminary experiments the practice of having no conversation save during the longer intervals was followed, but it was soon found that quiet talking about neutral themes tended to put the subjects at ease and to help them forget their breathing. Talking was not avoided, therefore, except during the time immediately before and during the actual tests. It is felt, too, that the series is reasonably free from errors due to excitement or discomfort. Some experiments were excluded because there was such evidence which seemed to make them unreliable.

The air samples were analyzed for carbon dioxide in a Haldane apparatus

TABLE 2

Data regarding metabolism, CO_2 per minute. From typical experiments. (Computed from O_2 taken in, with R. Q. assumed as 0.81)

SUBJECT	EXPERIMENT	DATE	RECLINING CO_2	SITTING CO_2	PER CENT CHANGE	STANDING CO_2	PER CENT CHANGE
B	6	1-21	172.9	176.8	+2.2		
	9	1-28	172.5			214.9	+24.6
	83	6-23	143.9			183.5	+27.5
C	16	2-17	180.2	204.3	+13.4		
	22	3-3	169.5			177.0	+4.5
	30	3-24	165.8			192.8	+16.3
	34*	3-31	155.6	178.7	+21.3		
G	19	2-26	158.8	154.3	−2.8		
	27	3-19	143.2	152.9	+6.8		
	58	5-8	151.8			168.9	+11.5
H	20	2-27	147.2	146.9	−0.2		
	24	3-13	144.3	145.6	+1.0		
	31	3-25	144.6			177.3	+22.6
O	43	4-13	153.6	160.5	+4.5		
	46*	4-16	149.2	163.0	+9.2		
	52	4-25	141.0			176.9	+25.5
P	45	4-15	164.8	158.8	−3.6		
	47*	4-17	150.6	165.1	+9.6		
	55	5-2	156.9			165.9	+5.7
Q	48	4-18	163.6	169.0	+3.4		
	49*	4-20	160.6	185.8	+15.7		
	59	5-9	164.4			192.3	+17.0
T	87	6-26	165.2	175.7	+6.4		
	90	6-29	155.3	161.6	+4.1		
	91	6-30	164.0			183.6	+12.0
W	93	7-2	155.1	157.0	+1.2		
	100	7-8	152.0	148.6	−2.2		
	108	7-15	148.6			163.2	+9.8
Y	104	7-11	129.4	132.4	+2.3		
	107	7-14	131.4	139.0	+5.8		
	109	7-16	130.6			157.6	+20.6
Averages for 25 subjects.....			149.6	158.7	+5.9	168.7	+13.6

*Experiments thus marked included a sitting period on a stool instead of a chair.

by the usual technic, and the results of the whole experiment were calculated by the method of Field and Bock (1924).

DISCUSSION OF RESULTS. The results of the experiments will first be taken up point by point and then in regard to their general significance.

1. Metabolism. Typical data on metabolism are shown in table 2. The basal metabolisms were in fair accord with existing standards, though the tendency to run rather low is in harmony with the opinion of Macleod and Rose (1925) and Macleod, Crofts and Benedict (1925), that standards for women may be 5 per cent too high. Four cases are more than 10 per cent below the Harris-Benedict standard. Two of these subjects have previously received treatment for low thyroid, though in neither case is there any disability to do the usual work; a third has had no reason to suspect a low thyroid condition; and the fourth was a subject who had had hundreds of basal metabolism tests made on her as a typical normal subject. Though this series was rather low for her it was not beyond the range of her other records. There are two cases where experiments on a given subject vary widely on different days. One of these showed two days about alike in January and a much lower set in June. This is of interest because of the problem of seasonal change in metabolism. Lindhard (1912, 1913a) found the basal metabolism higher in winter than in summer and the same is reported by Hafkesbring and Collett (1924) for a very different latitude, New Orleans versus Copenhagen. They attribute the difference to an effect of light. Benedict in an unpublished comment mentioned data which suggest a fall in basal metabolism as the work of the year goes on, from early autumn after the stimulation of a summer vacation to late spring. The results of this series which show unexpected variations are in harmony with an early summer decrease, whatever be its cause.

The rise in metabolism on sitting as compared with reclining averages 5.9 per cent, with a range from −6.8 to +24.9. The nine experiments where the subjects sat on a stool are averaged with the rest of the sitting experiments since the circulatory results were indistinguishable. The metabolism in these nine experiments, however, was distinctly higher, averaging 15.5 per cent more than in the reclining position, while the experiments in which the chair was used if averaged by themselves show an increase over reclining of only 3.3 per cent. The change on standing is an average increase over reclining of 13.6 per cent with a range from 0.8 to 27.5 per cent. These changes are in reasonable accord with other determinations of the same sort (Benedict and Benedict, 1924).

2. Alveolar air determinations. Table 3 shows a series of typical CO_2 determinations of alveolar and virtual venous samples and their difference. See Field, Bock, Gildea and Lathrop (1924), for details of calculation. The CO_2 in cubic centimeters is found by applying to the dissociation curve

TABLE 3

Data from typical experiments showing the difference (Diff.), minus 1 mm., between alveolar (Alv.) and virtual venous (V.V.) CO_2 tensions, in reclining, sitting and standing positions

SUBJECT	EXPERIMENT	DATE	RECLINING			SITTING			STANDING		
			Alv.	V.V.	Diff.	Alv.	V.V.	Diff.	Alv.	V.V.	Diff.
C	16	2–17	37.57	44.03	5.46	36.72	43.81	6.09			
			37.85	43.95	5.10	36.94	43.82	5.88			
			38.34	43.69	4.35	37.64	44.47	5.83			
	22	3–3	41.06	46.32	4.26				36.57	44.45	6.88
			41.33	47.12	4.80				36.33	44.52	7.19
			42.22	47.70	4.48				35.59	44.94	8.35
			41.74	47.73	4.99				29.53	43.90	13.37
	30	3–24	38.40	44.20	4.80				31.62	39.80	7.18
			38.78	44.40	4.62				31.40	40.49	8.09
			39.67	44.75	4.08				30.20	39.19	7.99
									27.13	38.00	9.87
	34*	3–31	41.54	46.63	4.09	36.75	44.13	6.38			
			41.30	47.05	4.75	37.13	44.33	6.20			
			41.67	47.02	4.35	37.15	44.45	6.30			
			41.80	47.03	4.23	36.67	44.04	6.37			
P	45	4–15	38.98	47.57	7.59	39.28	46.76	6.48			
			39.45	48.08	7.63	39.48	46.50	6.02			
						39.85	46.67	5.82			
						39.05	46.50	6.45			
	47*	4–17	38.56	46.28	6.72	36.27	44.60	7.33			
			38.80	46.52	6.72	36.53	43.97	6.44			
						36.94	44.20	6.26			
	55	5–2	40.05	47.32	6.27				35.92	43.28	6.36
			40.28	47.02	5.74				35.49	43.22	6.73
			39.70	47.38	6.68				35.43	43.35	6.92
			39.40	46.96	6.56						
T	87	6–26	40.03	46.23	5.20	39.16	46.32	6.16			
			40.17	45.90	4.73	38.34	45.70	6.36			
			39.95	45.48	4.53	38.52	44.80	5.28			
	90	6–29	41.05	47.00	4.95	38.00	46.05	7.05			
			41.40	47.40	5.00	37.91	45.80	6.89			
			41.23	47.25	5.02	38.37	45.95	6.58			
						37.50	45.85	7.35			
	91	6–30	41.42	47.00	4.58				35.72	41.80	5.08
			41.07	47.51	5.44				35.10	42.28	6.18
			41.10	46.70	4.60				32.22	42.98	9.76
									32.10	42.40	9.30
Averages for 25 subjects			40.22	46.63	5.42	38.26	45.65	6.39	35.06	43.87	7.77

*Experiments thus marked included a sitting period on a stool instead of a chair.

for normal individuals the tension in millimeters of Hg derived from analysis and corrected for barometric reading. Since these were all normal individuals the error introduced by the use of a standard curve seemed not greater than that likely to creep into determinations made by blood analysis, especially when the difficulty of getting samples which approximate mixed venous blood is considered. When the difference between arterial and virtual venous samples is found, it will be recalled that there is a subtraction of 1 mm. to allow for the difference in CO_2 content between the completely oxygenated blood of the test and the 95 per cent saturation of the usual arterial blood, and to allow also for the difference between alveolar air and arterial blood.

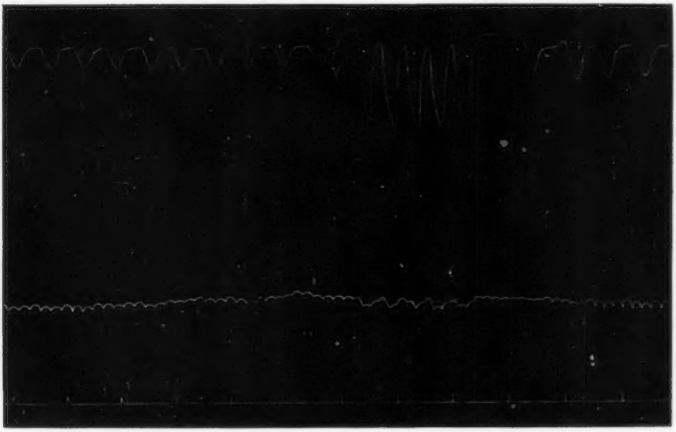

Fig. 1. Kymograph record of respiration and pulse during collection of Field-Bock sample. Respiration, top line; heart-rate, middle line; time line with 5-second intervals below. Note that subject's respiration is even before sample is taken. The alveolar sample is taken at the end of a normal respiration. The five deep breaths from the bag of O_2 and CO_2 take less than 15 seconds.

Figure 1 is a portion of a kymograph record showing the way in which the respiration is followed during the collection of the Field-Bock samples. The preliminary respiration is even. The point at the end of a normal respiration at which the forced respiration is made for the alveolar sample is clearly shown as are also the successive deep breaths from the bag. The time record with five second intervals shows that the whole affair is over before there is danger of return of blood which by recirculation has been further loaded with CO_2, since the fifteen seconds of the test are well under the probable time for a complete double circulation. When these respiration records showed that the few breaths before the collection of the sample

were either deeper or more shallow than usual, the variations in the amount of CO_2 in the samples were in harmony so that it became possible to use the kymograph records as a control. Samples were therefore discarded as has been said when the preliminary breathing or the breathing during the test was unsatisfactory. Such cases were not infrequent, though in the whole series of over seventy experiments, each with eight tests, there were very few instances where the results of a determination depend on the averaging of less than three samples, and in many cases all four were used.

For the reclining position, successive alveolar samples were expected to check within 1 mm. CO_2 tension. When cases less close than this are included it is because the differences between successive arterial and virtual venous samples were so small as to make their accuracy probable.

It was easily apparent that the subjects in the sitting and still more in the standing position show less regularity in breathing than when reclining. This may have been due in some cases merely to restlessness incident to standing still when attached to the apparatus and breathing through the mouthpiece, though everything was made as comfortable as was possible. But certainly in the standing position and in some instances in the sitting position in addition to less even breathing there is a shift in CO_2 values, too regular to make casual variation a sufficient explanation or even a probable one. This lowering of the alveolar CO_2 seems to be related to the very phenomenon being studied, the disadvantage at which the circulation is placed when the body is erect. Higgins (1914) notes the same change as was found in this series of experiments, a lowering of alveolar CO_2 when standing or whenever there is a "sense of effort." The sense of effort is experienced by some subjects even when sitting still, as was evidenced by the obvious relief when it was possible to move about at the end of the experiment, and this in spite of a very comfortable sitting position. For nearly all subjects the lowering of alveolar CO_2 was evident in standing, and the subjects who show this lowering most markedly are the ones who stood least successfully both subjectively and objectively. Note subject C, for whom the alveolar air dropped conspicuously in both standing experiments. She was so dizzy and faint in experiment 30 that it was with difficulty that the series of four tests was completed. She all but fell as the mouthpiece was taken out at the end of the last test. It is evident from the regularity with which the fall in alveolar CO_2 occurs and from its progressive nature that this is no chance overbreathing but a real reaction to the conditions of the position.

3. Virtual venous air determinations. This term is used, following Y. Henderson (1922) and Field, Bock, Gildea and Lathrop (1924), for the CO_2 content of the gas samples from the bag, gas which is in CO_2 equilibrium with the mixed venous blood entering the lungs. These determina-

tions showed a most pleasing consistency and dependability, indicating a good CO_2 equilibrium between gas and venous blood.

The virtual venous CO_2, like that of the alveolar air, fell with the change from reclining to standing; in other words, ventilation increased more rapidly than the need for it. In good experiments the successive tests in the reclining position gave differences between alveolar and virtual venous CO_2 which checked very closely. These varied somewhat more in sitting and still more in standing, but the change was not a random fluctuation.

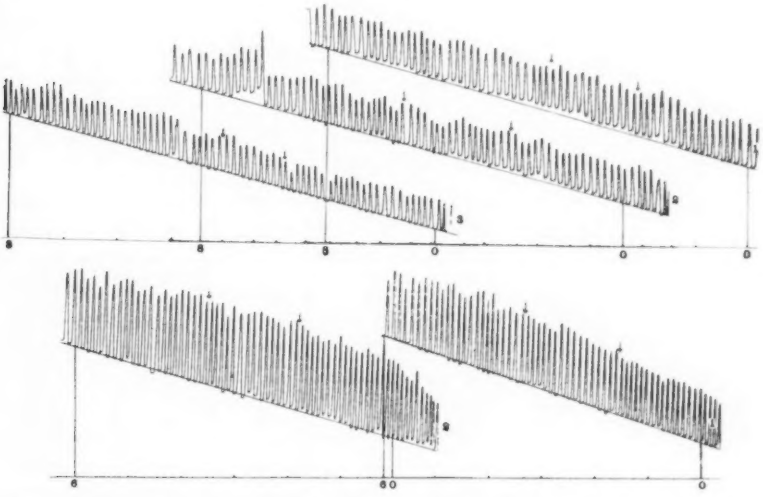

Fig. 2. Kymograph records of reclining and standing respiration during metabolism determinations by Benedict-Roth apparatus. Records read from right to left. Upper records are of reclining, lower of standing respiration. Numbers below the line indicate beginning and end of the several 8- and 6-minute periods of the tests. Arrows mark the beginning and end of tests for leaks in the apparatus. Note that ventilation in standing shows a marked increase in both rate and depth of breathing. Average rate per minute: reclining, 7.8 breaths; standing, 9.2 breaths.

A definite progression is apparent in many experiments as in the lowering of the alveolar CO_2. The longer the set of experiments was continued and the more carefully the possible causes of error were eliminated, the more definite became the conviction that these changes are real changes induced in the blood by the maintenance of a quiet erect position.

In only one experiment in the whole series do both alveolar and virtual venous CO_2 rise; in a few one or the other rises very slightly, but in nearly all both alveolar and virtual venous CO_2 show a fall, more marked in the alveolar CO_2, as has been said.

TABLE 4

Data showing the change in circulatory minute volume (C. M. V.), heart rate (H. R.), and the output per beat (O. p. B.) from the reclining to the sitting and standing positions. Schneider ratings are also given

SUBJECT	EXPERIMENT	DATE	RECLINING			SITTING						STANDING						SCHNEIDER RATING
			C.M.V.	H.R.	O.p.B.	C.M.V.	Per cent Ch.	H.R.	Per cent Ch.	O.p.B.	Per cent Ch.	C.M.V.	Per cent Ch.	H.R.	Per cent Ch.	O.p.B.	Per cent Ch.	
A	8	1-27	6.06	53.0	114.7	4.76	−21.4	58.0	+9.5	82.2	−28.2							13
	12	2-3	6.21	65.3	95.1							5.83	−6.2	74.3	+13.8	78.5	−17.5	11
B	6	1-21	5.57	64.5	86.3	6.31	+7.7	71.3	+10.5	88.5	−2.6							14
	9	1-28	6.39	62.0	103.0							8.21	+28.5	76.0	+22.5	108.0	+4.8	13
	83	6-23	5.21	55.0	94.7							4.45	−14.6	79.5	+44.6	51.9	−45.2	15
C	16	2-17	7.72	81.5	94.6	7.33	−5.1	86.0	+5.5	85.2	−9.9							
	22	3-3	8.12	73.5	110.5							4.12	−49.2	99.?	+34.7?	41.6?	−62.3?	6
	30	3-24	7.76	79.3	98.0							4.56	−41.2	88.7?	+11.8?	51.4?	−47.5?	
	34*	3-31	7.94	77.5	102.5	6.02	−24.2	92.0	+18.7	65.5	−36.1							12
D	26	3-18	4.39	58.0	75.7	4.69	+6.8	66.5	+14.6	70.5	−6.8							13
	64	5-21	4.03	53.3	75.6							3.53	−12.4	70.0	+31.3	50.4	−33.3	15
E	21	2-28	5.07	56.5	89.8							4.80	−5.4	73.5	+30.1	65.3	−26.9	
	25	3-14	4.79	60.0	79.8	4.20	−12.2	67.5	+12.5	62.2	−22.0							10
F	17	2-18	7.15	69.0	103.6	7.03	−1.7	72.0	+4.4	97.7	−5.7							13
	23	3-11	6.90	74.0	93.2							4.65	−30.6	94.5	+27.7	49.2	−47.2	10
G	19	2-26	7.43	57.0	130.5	7.18	−3.3	57.5	+0.9	124.9	−4.3							15
	27	3-19	7.86	53.5	146.8	7.86	0.0	57.0	+6.5	137.9	−6.0							16
	58	5-8	7.61	60.0	126.6							7.48	−1.7	91.0	+51.7	82.2	−25.1	

H	20	2–27	7.57	69.0	109.7	4.38	−42.1	74.0	+7.3	59.2	−46.1							10
	24	3–13	5.37	60.0	89.4	5.26	−2.0	74.0	+23.3	67.9	−24.1							12
	31	3–25	6.47	66.0	98.1							2.92	−54.9	98.0	+43.4	29.8	−69.3	9
I	29	3–23	6.88	68.0	101.2	8.55	+24.5	84.5	+24.2	101.2	0.0							
	32	3–26	5.99	71.0	84.4	5.37	−10.3	74.7	+5.2	71.8	−14.9							13
	88	6–27	6.24	66.0	94.5							5.35	−14.3	79.0	+19.7	67.8	−28.2	13
J	42*	4–11	6.41	55.3	115.9	7.02	+9.5	60.0	+8.5	117.0	+1.0							15
	81	6–19	6.23	63.5	98.2							5.87	−5.8	102.0	+60.6	57.5	−41.4	10
K	35	4–1	6.16	73.0	84.3	5.03	−18.5	76.0	+4.0	66.1	−21.5							9
	40*	4–8	5.96	77.0	77.4	6.47	+8.5	88.7	+15.2	72.9	−5.8							12
	53	4–29	6.74	80.0	84.3							4.83	−28.3	89.0	+11.2	54.3	−35.6	11
L	36	4–2	7.49	70.0	107.0	6.23	−16.9	75.0	+7.1	83.1	−20.4							12
	38*	4–6	6.66	62.0	105.8	5.53	−17.0	75.3	+21.5	73.4	−30.6							12
	51	4–24	6.69	58.0	115.4							4.37	−34.7	91.0	+51.8	48.0	−58.4	16
M	37	4–3	5.57	70.0	79.5	4.06	−27.1	72.5	+3.5	56.0	−29.5							11
	39*	4–7	6.59	63.5	103.8	5.40	−18.1	70.7	+11.4	76.3	−26.5							14
	54	5–1	4.80	59.3	81.0							4.36	−9.0	86.5	+45.9	50.5	−37.6	12
N	41	4–9	6.24	69.3	90.1	4.85	−22.3	74.0	+6.7	65.5	−27.3							13
	44*	4–14	4.77	64.0	74.6	4.87	+2.1	72.7	+13.6	67.0	−10.2							15
	50	4–23	5.86	62.7	93.4							3.51	−40.1	87.0	+38.8	40.3	−54.9	15
O	43	4–13	6.79	71.0	95.6	7.19	+5.8	65.0	−8.4	110.5	+15.7							11
	46*	4–16	6.26	66.0	94.8	5.91	−5.6	68.0	+3.0	86.9	−8.3							12
	52	4–25	5.69	60.5	94.1							5.15	−9.5	69.0	+14.0	74.6	−20.7	

*Sitting on stool.

TABLE 4—*Concluded*

SUBJECT	EXPERIMENT	DATE	RECLINING			SITTING						STANDING						SCHNEIDER RATING
			C.M.V.	H.R.	O.p.B.	C.M.V.	Per cent Ch.	H.R.	Per cent Ch.	O.p.B.	Per cent Ch.	C.M.V.	Per cent Ch.	H.R.	Per cent Ch.	O.p.B.	Per cent Ch.	
P	45	4–15	4.87	61.0	79.8	5.60	+15.0	64.0	+5.0	87.5	+9.6							15
	47*	4–17	4.87	58.0	84.0	5.26	+8.0	71.0	+22.4	74.1	−11.8							15
	55	5–2	5.52	61.0	90.5							5.24	−5.1	88.0	+44.2	59.5	−34.2	15
Q	48	4–18	5.87	64.0	91.8	5.16	−12.1	66.0	+3.1	78.2	−14.8							17
	49	4–20	5.20	60.0	86.7	4.30	−17.3	67.0	+11.7	64.2	−26.2							18
	59	5–9	4.41	61.0	72.2							3.69	−16.3	86.5	+41.8	42.9	−40.6	17
R	57	5–6	9.48	62.5	151.6	8.44	−10.9	68.0	+8.8	124.4	−18.1							14
	63	5–20	6.42	61.3	104.7							6.33	−1.4	83.0	+35.4	75.3	−28.1	13
S	56	5–5	4.90	67.0	73.1	3.34	−32.2	74.0	+10.4	44.8	−34.3							
	60	5–12	5.29	57.0	92.9	4.44	−16.0	61.0	+7.0	73.8	−20.5							16
	62	5–19	4.83	62.0	77.9							3.48	−27.9	80.0	+29.0	43.5	−44.1	16
T	87	6–26	7.50	68.0	110.3	6.40	−14.7	67.3	+1.0	95.1	−13.8							8
	90	6–29	6.92	66.7	103.7	4.99	−27.8	73.0	+9.5	68.3	−34.1							9
	91	6–30	7.56	64.7	116.8							5.08	−32.8	96.5	+49.1	52.6	−55.0	13
U	95	7–3	7.74	82.5	93.9	5.93	−23.4	95.0	+15.1	62.4	−33.5							5
	98	7–7	7.90	80.5	98.2	6.30	−20.2	93.5	+16.1	67.4	−31.4							7
	101	7–9	7.44	74.0	100.5							5.91 incomplete	−20.5	96.0	+29.7	61.5	−38.8	14?

V	96	7–6	6.49	65.0	99.8	4.60	−29.1	69.3	+6.6	65.8	−34.1						15
	102	7–10	5.99	62.5	95.9	4.27	−28.7	68.5	+9.5	62.3	−35.0						16
	110	7–17	7.47	66.0	113.2							3.67	−50.7	83.3	+26.2	44.1	−61.0	13
W	93	7–2	6.94	64.5	107.6	5.45	−21.5	80.0	+24.0	68.1	−36.7						11
	100	7–8	8.48	67.5	125.7	6.55	−22.7	73.3	+8.6	89.3	−29.0						12
	108	7–15	6.97	59.0	118.1							4.27	−38.7	72.7	+23.2	58.2	−50.7	14
X	92	7–1	5.76	57.5	100.2	5.43	−5.8	62.7	+9.1	86.6	−13.6						15
	106	7–12	5.49	52.5	104.5	4.95	−9.8	59.5	+13.3	83.1	−20.5						11
	112	7–21	5.41	57.0	95.0							4.01	−25.9	76.5	+34.2	52.4	−44.8	10
Y	104	7–11	4.36	66.0	66.1	2.95	−32.3	77.3	+17.1	38.3	−42.0						13
	107	7–14	4.07	60.5	67.3	4.72	+15.8	65.0	+7.4	72.6	+7.8						15
	109	7–16	4.15	63.0	65.9							3.13	−24.6	86.0	+36.5	36.4	−44.8	16
Average			6.26	64.7	96.9	5.59	−10.9	71.8	+10.1	79.0	−18.2	4.77	−21.2	85.1	+33.4	56.6	−40.3	

*Sitting on stool.

It may thus be possible that an oxygen factor enters into the control of respiration in the longer standing experiments, in those cases in which the fall of the alveolar CO_2 is most marked, for these are the cases in which the subjects showed other signs of discomfort.

The same tendency to increase the respiratory minute volume is noted when the graphic records of standing and reclining metabolism are studied. See figure 2, where the kymographic records of reclining and standing metabolism tests on one subject, taken in one forenoon, are reproduced. The elevation of metabolism in the standing position in this case was 20.6 per cent above that obtained for the reclining position, but the ventilation is obviously increased more than this. Other experiments, to be reported in another article, indicate the probability of lessened return of blood as a cause of the circulatory changes found. This puts the standing individual in the group of those in whom the circulation may easily become inadequate. Peters and Barr (1921) found that in cardiac subjects the difference between alveolar and arterial CO_2 tension is greater than in normal subjects, an effect produced by lowering the alveolar CO_2. They consider that this is due to the need of facilitating the escape of the necessary amount of CO_2 when the circulation is impaired and the lung conditions thus not normal. The same reason might hold in these cases where the heart is not defective and where, though there is no reason to suspect lung inadequacy, the diminished return of blood to the heart gives a circulation inadequate to the demands upon it. Further, the unfavorable position of the respiratory center, high above the heart, is to be noted. Plesch (1913) in a study of the mid-capacity of the lungs considers the condition of the right heart favored by reclining rather than by the erect position. All this implies the desirability of using, contrary to much medical practice, the reclining rather than the sitting position as a standard, an assumption which seems also to rest on secure evolutionary foundation.

4. Circulatory minute volume. Table 4 gives the data retarding circulatory minute volume, heart rate and output per beat in the whole series of seventy experiments. The ascertaining of these data was the chief end of the experiments. Figures 3, 4, 5 and 6 also give in more graphic form data regarding the changes in minute volume.

The average value for the circulatory minute volume for all the reclining experiments is 6.26 litres, with a range from 4.03 to 9.48, figures obtained from one of the shortest and one of the tallest subjects respectively. In general the reclining values from one individual obtained on different days are in fair accord though there are some exceptions.

The findings for the forty-three sitting experiments show an average value of 5.59 litres, a fall below the reclining average of 10.9 per cent. These experiments showed a rise in ten instances, a negligible change in

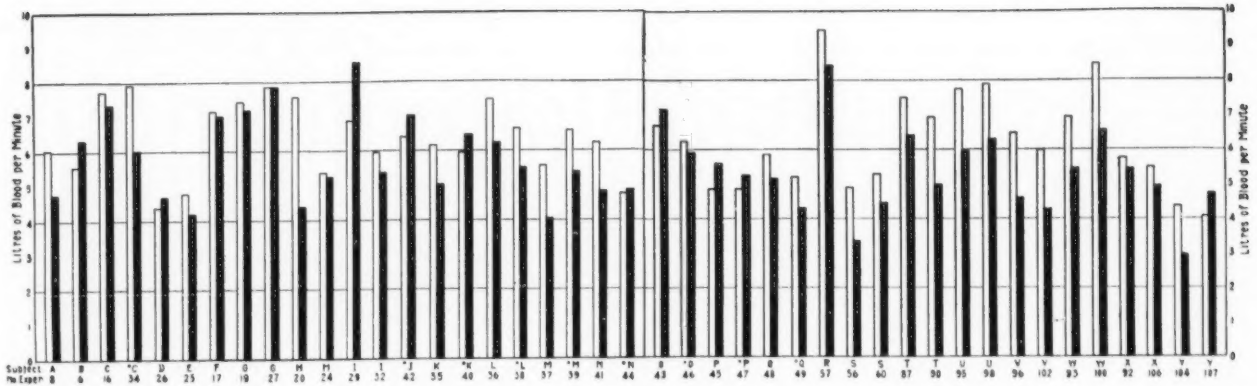
Litres of Blood per Minute
Litres of Blood per Minute
Subject: A B C *C D E F G G M M I I *J K *K L *L M *M M *N B *O P *P Q *Q R S S T T U U W Y W YY R X Y Y
Reclining: B 0 16 36 26 25 17 10 27 20 24 29 32 42 55 40 56 38 37 39 41 44 43 46 45 47 48 49 57 56 60 87 90 95 98 96 102 93 100 92 106 104 107

Fig. 3. Circulatory minute volumes. Comparison of actual volumes in reclining and sitting positions. In experiments marked * the subject sat on a stool; in all others, in a chair. Blank columns, reclining volumes; black columns, sitting volumes.

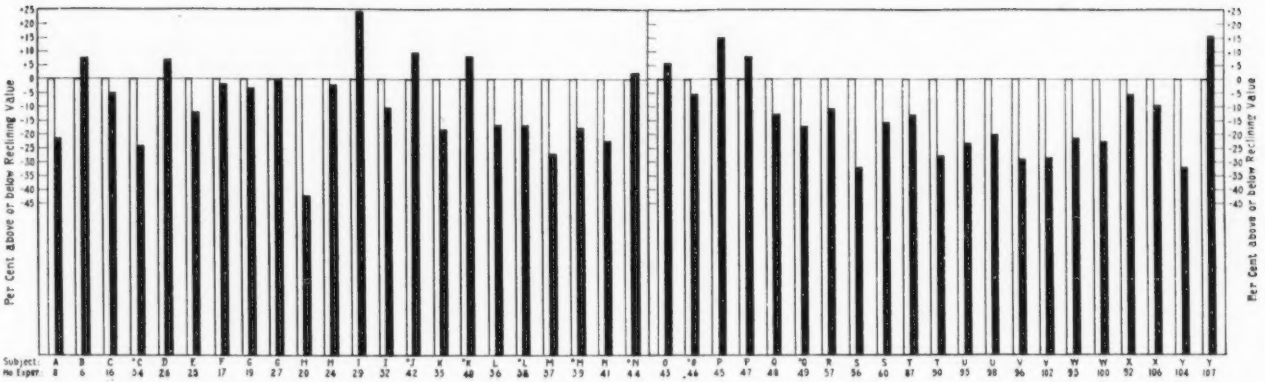

Fig. 4. Circulatory minute volumes. Percentage relations in reclining and sitting positions, with reclining volume taken as 100 per cent. Symbols as in figure 3.

Fig. 5. Circulatory minute volumes. Comparison of actual volumes in reclining and standing positions. Blank columns, reclining volumes; black columns, standing volumes.

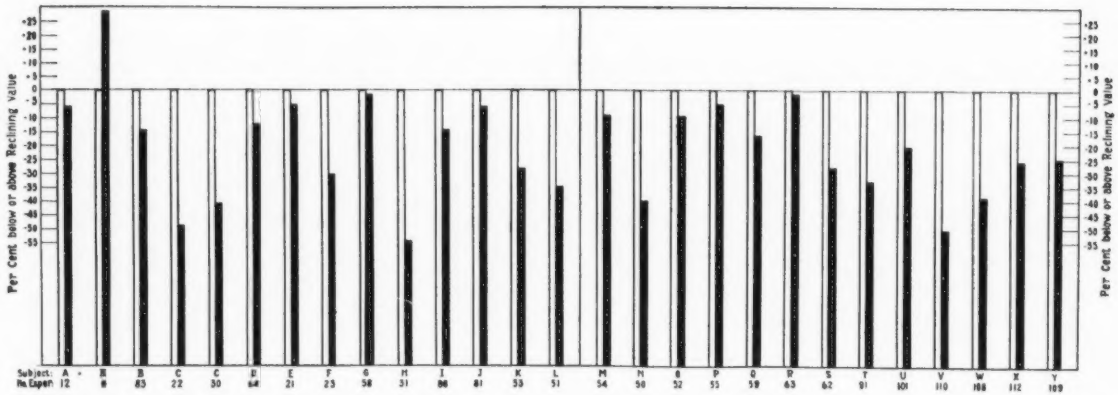

Fig 6. Circulatory minute volumes. Percentage relations in reclining and standing positions, with reclining volume taken as 100 per cent. Symbols as in figure 5.

two and a fall from 2 to 42 per cent in the remaining large number of experiments, thirty-one. The metabolism, it will be recalled, increased by 5.9 per cent. It is thus seen that in sitting the circulatory minute volume tends strongly to fall, while the metabolism rises, a finding directly at variance with the results on exercise previously referred to. Testimony is united that the volume of the heart's output follows very closely the increasing demands of the organism in exercise. That the metabolism increases on sitting there is further evidence in the increased heart rate, from an average of 64.7 reclining to 71.8 sitting, figures quite in harmony with those of many observers and which tend to show that these subjects were reacting in a normal way. The output per beat thus falls markedly, from an average of 96.9 cc. reclining to 79.0 cc. sitting.

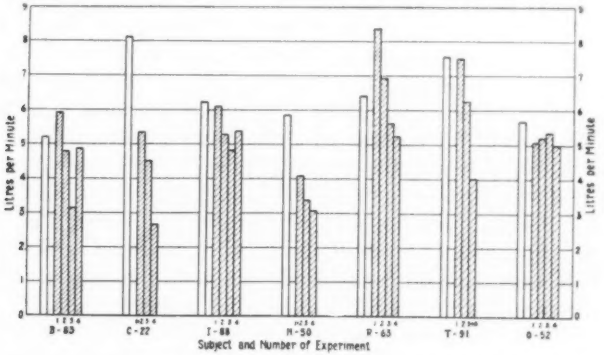

Fig. 7. Circulatory minute volumes in seven typical standing experiments showing variations as standing continued. Blank columns, reclining volumes; hatched columns marked *1*, *2*, *3*, *4*, standing volumes in successive periods. Two periods with volumes closely alike were averaged and marked *1* and *2*, etc.

The same tendencies are shown in the series of twenty-seven standing experiments, but to a degree much more marked. The average circulatory minute volume is 4.77 litres, a decrease of 21.2 per cent from the reclining value. The percentage fall ranges from −5.8 to −54.9 in twenty-four experiments, while the other three experiments show a negligible change in two cases and a real rise in only one, +28.5 per cent. This last experiment was on a tall, vigorous young woman of excellent posture. The experiment was repeated on her after a lapse of five months, but on the second occasion though the two Field-Bock determinations, corresponding to minutes 3 and 6 after rising, showed an increase in the minute output, she was unable to continue this output for the last half of the test in which there was a distinct fall, giving an average for the whole series of −14.6 per cent. The data for the first standing experi-

ment seemed trustworthy, however, so it was not left out of the series even though it is conspicuously alone. The average standing metabolism increases by 13.6 per cent above the reclining value, the average heart rate increases by 33.4 per cent from 64.7 to 85.1 beats per minute. The average output per beat thus goes down very markedly, from 96.9 to 56.6 cc., or −40.3 per cent.

The picture of a falling minute volume as the quiet erect position is maintained is thus convincing. Further details of this appear when individual experiments are studied more closely. The minute volumes referred to above are averages of each subject's set of samples for a given experiment. If the history of a given subject is studied for a period of quiet standing it is found that the change is very often progressive rather than fluctuating and that therefore the readings really should not be averaged at all. Experiment C-22 (see fig. 7) shows the results on a subject whose whole record gave a picture of marked decrease in output on taking the erect position. She was the subject previously referred to who on one occasion was hardly able because of faintness to complete the tests. The record for experiment C-22 shows a fairly steady condition for standing periods 1 and 2, a time of about eight minutes, though the output was 5.35 litres as contrasted with 8.12 litres found in the reclining position. Periods 3 and 4 show a pronounced fall, reaching the very low value of 2.95 litres after about fourteen minutes of quiet standing. Contrasted with this poor result note 0–52, a most successful experiment, where there were only very slight changes between the successive standing determinations. Subject O was a student from the school of Physical Education and stood in beautiful position, with perfect ease. She was entirely comfortable and unwearied at the close of the experiment. The case of I-88 was interesting. At the end of period 3 the subject said she could not complete the experiment because of an irritating itching sensation and stamped her foot two or three times. She was urged to keep on for the last test and did so, with the result shown in the chart, a rise in the last period whereas there had been previously a gradual fall during her time of quiet standing. It is easy to think that her restlessness and few rhythmic movements had helped the return of blood from the lower part of the body enough to give the result shown in the last test.

When the twelve experiments in which this progressive change on prolonged standing shows most clearly are segregated and their *lowest* results averaged, the figure of 3.53 litres per minute is obtained, a fall of 39.6 per cent from the standard reclining figure of 6.26. The average pulse for these low periods is above that of the general standing average, 90.9 as compared with 85.1 and the output per beat 39.3 cc. versus 56.6 cc. The conspicuous rise in the heart rate of these subjects is to be noted and will be discussed in a later study.

FACTORS INFLUENCING MINUTE VOLUMES. *1. Stature.* An effort was made to find out whether height is related to the heart's output per minute. The number of subjects was too small, of course, for satisfactory statistical results to be expected. When the subjects are arranged by height, the obviously poor and especially good subjects scattered irregularly through the group altered the average for their neighborhood markedly; yet it is true that in a series including all reclining volumes the average for the subjects who are taller than the mean height of 1150 Wellesley freshmen (162.9 cm.) is 6.59 litres while the average for the group shorter than this mean height is 5.59 litres, variations from the average for the whole group of +4.2 per cent and −10.7 per cent respectively. In the taller group are 17 subjects, involving a total of 50 experiments; in the shorter group 8 subjects involving 20 experiments. It is suggested therefore, that stature has an effect on the circulatory minute volume.

2. Training. Three of the subjects from the Boston School of Physical Education, O, P and Q, were in excellent training, near the end of the second year of their course. The fourth subject of this group became seriously ill shortly after the experiments so her record which is less good than that of the others may not be suggestive of the effect of training. The three subjects O, P and Q have an average reclining circulatory minute volume of 5.50 litres. Their average height is 165.5 cm., whereas the average height of the whole group is 163.8. They are thus taller but with lower reclining minute volumes, evidence, as far as it goes, in favor of a low minute volume associated with training. They all gave records of adjustment to the erect position above the average in excellence as is evidenced by the table below.

	SITTING		STANDING
	Chair	Stool	
	*per cent**	*per cent*	*per cent*
Subject O	+5.8	−5.6	−9.5
Subject P	+15.0	+8.0	−5.1
Subject Q	−12.1	−17.3	−16.3

* Per cent in each case indicates decrease or increase over reclining value.

Subject M is also worthy of special mention, because of her previous experience as an artist's model and hence her being accustomed to holding any desired position. Her usual routine was to pose for 25 minutes and to rest for 5 minutes. For the last few years, since her marriage, she has been very frequently though not continuously a subject for metabolism research in which she has had many standing metabolisms taken. She was a most dependable subject in the constancy of her determinations. She seemed muscularly very "soft" and not at all energetic in manner or

movement, a great contrast to the girls in physical training. Her records were these, poor for the sitting position, but good for standing.

	SITTING		STANDING
	Chair	Stool	
	per cent	*per cent*	*per cent*
Subject M	−27.1	−18.1	−9.0

Subjects who were used to standing in the laboratory but who had had no formal physical training do not show outstandingly good records. As far as the evidence goes, it would seem that training may have an effect.

3. Season. During none of the actual experiments were the room conditions uncomfortable for the subjects. In the summer they had the best possible breeze and in the winter they had blankets to compensate for their enforced quiet in a room of ordinary temperature.

The case of subject B whose power of adjustment was much poorer in June than in January has been mentioned. The effect of temperature as such does not appear in this series, though averages of winter and summer experiments give somewhat larger volumes in the reclining position for the latter. This is in harmony with Barcroft and Marshall (1923). It may be noted also that of the six subjects (T, U, V, W, X, Y) whose experiments were done late in June and in July, none showed a good record for adjustment to the erect position. The results follow.

SUBJECT	SITTING	STANDING
	*per cent**	*per cent*
T	−14.7	−32.8
	−27.8	
U	−23.4	−20.5†
	−20.2	
V	−29.1	−50.7
	−28.7	
W	−21.5	−38.7
	−22.1	
X	−5.8	−25.9
	−9.8	
Y	−32.3	−24.6
	+15.8	

* Per cent in each case indicates increase or decrease over reclining value.

† Experiment incomplete. Final result probably would have shown greater fall.

The following series was completed before the weather became warm. Subject C is omitted because of her uniformly very poor record, and one experiment of B because done late in June.

SUBJECT	SITTING	STANDING
	per cent	*per cent*
A	−21.4	−6.2
B	+7.7	+28.5
D	+6.8	−12.4
E	−12.2	−5.2
F	−1.7	−30.6
G	−3.3	−1.7
	±0.0	

In considering the physical fitness of these two groups, it is to be noted that T and U in the "hot weather" group showed other indications of a circulation none too dependable but the other four were presumably thoroughly good subjects and both W and X had been playing tennis all through the spring. Of the "cold weather" group, F was not a good subject as brought out by other evidence, while B was apparently the only really sturdy one in the group, yet their results seem much better than those of the summer group. "Season" may be a complex thing made up not only of heat and humidity but of the fatigue of the working year and other factors. The general trend of the summer results is distinctly toward a lessened power at that season to compensate for the erect position. Dilatation of the peripheral vessels may be a factor in this.

GENERAL DISCUSSION. While the zone of error from inaccuracies of method and unavoidable personal factors is undoubtedly a large one, nevertheless the results in general are sufficiently consistent to make a strong case for a fall in circulatory minute volume on changing from a reclining position to one of quiet standing. This harmonizes too well with the ordinary experiences of life to make it open to serious question, even though it is at variance with the needs of a rising metabolism. The outstanding feature of the occasions in ordinary life when this circulatory embarrassment appears is the *quiet* maintenance of the erect position. The fainting of West Point cadets at inspection, the fainting of women whose clothes are being fitted, the collapse of persons who stand for a considerable time to see parades, to listen to speeches in crowded auditoriums, these are frequent examples. Heat and humidity increase the liability to faint. Many persons including some of those in this group know that they are apt to grow dizzy or to faint if they stand for any considerable period. Some of them have definite ways of trying to protect themselves which amount to helping the blood back to the heart by exercise, as by shifting the weight rhythmically, contracting and

relaxing the leg muscles, and, in general, by making as extensive movements as they can make undetected, for they are usually ashamed of their limitations. Some take deep breaths frequently, thus increasing the aspiration of the thorax. When the whole series of phenomena is scrutinized, the evidence for a decreased return of blood becomes impressive.

What becomes of the blood which ought to be returning to the heart does not appear in these experiments, nor whether it is an arterial or a capillary phenomenon. Sewall (1916, 1919) places great emphasis on the possibility of stagnation in the mesenteric vessels and the liver, with the idea, too, that the tone of the abdominal musculature is of great importance for a proper return of blood, and that ptosis is associated with a failure of the mesenteric reflex whereby the diastolic pressure is usually and rightly raised on standing. The older work of Hill (1895) and of Hill and Barnard (1897) is in line with this. In the quiet standing position the blood has little to aid its return. Gravity is against the large amount below the heart, there is no rhythmic pumping of the locomotor muscles so often used in alternation, the exercise of standing increases the CO_2 in the blood too little to augment respiration largely and so facilitate thoracic aspiration; indeed everything is against the filling of the heart. That more liquid actually tends to accumulate in the legs is indicated by the observation of Bock who found that the legs increase in girth when standing is prolonged. The unexpected exaggeration of the respiration seen in these experiments, as noted in the section on alveolar air, may be of material assistance in getting more blood back by aspiration. Indeed this exaggeration of the respiration may be in its nature related to the dyspnea of inadequate circulation. The disproportionate increase in the rate of the heart over the requirements of metabolism is also in line with the idea of a failure in the return of blood.

The changes observed in pressure relations are in harmony with the explanation that the return of blood is lessened during quiet standing. If effective distribution of blood is to be maintained, a systolic pressure suitable for reclining surely should not fall on rising. Yet such a fall is often just what occurs, as is shown by a study to be reported later. Such a fall might naturally follow a diminished return of blood. The mean pressure of the erect position is however maintained at a level approximating that of the reclining position by a rise in diastolic pressure. This rise narrows the pulse pressure, a fact in harmony with the finding of a circulatory minute volume which is much decreased.

A lowered circulatory volume in the quiet erect position is not out of harmony with the evolutionary derivation of the erect from the horizontal animals. It is perhaps to be expected that the phylogenetically later position should show a less perfect adjustment, especially in view of its

intrinsically greater difficulty from the physical standpoint of the movement of liquid in vessels.

SUMMARY AND CONCLUSIONS

1. Circulatory minute volumes were determined by the Field-Bock method on 25 healthy young women in reclining, sitting and standing positions.

2. While the metabolism showed as usual a small rise on sitting as compared with reclining and a larger rise on standing, the minute volumes changed in the opposite direction, a fall averaging 10.9 per cent on sitting and 21.2 per cent on standing. The average values in litres for the three positions were, reclining, 6.26 litres; sitting, 5.59 litres; standing, 4.77 litres.

3. It appeared from a study of the details of the experiments that the difference between the CO_2 of the arterial and venous bloods became greater as standing was continued, a change associated with a fall in the CO_2 of the alveolar air, but that this was too regular and too frequently progressive to be a case of overventilation, a treacherous possibility which was further guarded against by care in technic.

4. The heart rate was increased in sitting as compared with reclining and further increased on standing. This latter increase was greater than the increase of the metabolism. The output per beat was thus decreased on sitting as compared with reclining and much more decreased on standing.

5. There are probably relations between circulatory minute volumes and stature, training and season. The essence of the hot weather handicap on the circulation may be the lessened return of blood to the heart due in turn to skin dilatation.

6. Supporting evidence is offered that such a lessened output from the heart on taking a quiet erect position is in harmony with the ordinary experience of many persons as shown by circulatory embarrassment of various kinds on prolonged and quiet standing. It is further in harmony with the relatively late phylogenetic development of the erect position.

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A STUDY OF ANION AND CATION EFFECTS ON WATER ABSORPTION BY BRAIN TISSUE

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Since Hofmeister's well-known experiments (first reported in 1888) a mass of literature has accumulated on the effects produced by various anions and cations on the physical properties of proteins. In the summary of the literature given by Höber (1) we find that the study of ion effects has been extended to a number of physiological processes, such as hemolysis, phagocytosis, muscle irritability, heart activity and nerve irritability. In their physiological effects the anions can be arranged, with a few minor exceptions, in the same series (the so-called Hofmeister series), which obtains for osmotic pressure, viscosity and swelling of gelatin: SO_4, tartrate, citrate < acetate < Cl < Br, NO_3 < I < CNS. For the swelling of gelatin the series is taken to read the greatest amount of swelling in CNS and the least in SO_4.

Although the differences in cation effects are not so well defined, the action of bivalent ions is unquestionably distinct from that of monovalent ions. As to the latter "it might be possible to propose the series Li < Na<K, NH_4; then follow the alkali earths with Mg in a position between." (Höber; cf. 2 and 3.)

A serious objection has been raised to the Hofmeister series by Loeb (4), who regarded the series as purely fictitious. In the experiments which led to its formulation, Loeb observed that no determination was made of the hydrogen ion concentration. From his own experimental studies, this investigator concluded that the effects which have been ascribed to the chemical nature of the ions must be attributed to variations in the hydrogen ion concentration. He held that it is not the chemical nature of the ion but the valency which accounted for different effects observed on the physical properties of proteins.

Proteins are amphoteric electrolytes and at a definite hydrogen ion concentration, the isoelectric point, can combine neither with anion nor cation. The isoelectric point of gelatin Loeb found to be pH 4.7. "At

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a pH > 4.7 gelatin can combine only with cations (forming metal gelatinate e.g., Na gelatinate); at a pH < 4.7 gelatin combines with anions (forming gelatin chloride, etc.)." Since the hydrogen ion concentration of the proteins was not controlled in the experiments which led to the formulation of the Hofmeister series, Loeb argues that the ions as such did not have specific effects on the physical properties of the proteins but that the proteins formed with the ions new compounds which have different physical properties than the original proteins. At the isoelectric point equal concentrations of the different sodium salts and the various chlorides of the same valency produce identical effects. " . . . . ions of the same valency and the same sign of charge have, in the same concentration, nearly the same depressing effect on osmotic pressure, swelling and the viscosity of proteins, while the depressing effect increases rapidly with the valency. The Hofmeister ion series are chiefly due to the failure to measure the influence of salts on the hydrogen ion concentration of the gelatin solutions and gelatin gels" (Loeb).

Since Loeb's experiments Gerngross and Bach (5) have shown that a wide variation occurs in the isoelectric point of different gelatins. In several samples of the purest commercial product, some of which were purified by electro-osmosis, the pH was found to vary from 4.7 to 5.5. Ostwald and his co-workers (6) studying the effect on the swelling of gelatin by different acids, determined electrometrically the iso-electric point of all the gelatin used in their experiments. Different acids of the same pH produced a characteristic effect on swelling represented as follows:

hydriodic > glycerolphosphoric > sulfosalicylic > hydrochloric > sulfuric

At a definite pH gelatin swelled more in dibasic than in monobasic acids, but in one instance the difference between the swelling in a monobasic and in a dibasic acid is less than in two different monobasic acids. These experiments, although not referred to the Hofmeister series, dispose, we think, of the objection raised by Loeb against a specific ionic effect on the hydration of proteins.

THE PROBLEM. It has been shown that in the several parts of the brain there is a marked difference in the rate and degree of water absorption (7). The present study was undertaken to determine (a) whether the ions of the Hofmeister series have a specific effect on water absorption by the cerebral hemispheres, cerebellum, mid-brain and medulla; (b) whether there exists among the different portions of the brain any relation in the effect of ions.

Hooker and Fischer (8) using in their experiments the entire rabbit's brain, formulated for the anions of the sodium salts the series:

$$Cl < NO_3 < \text{acetate} < \text{phosphate} < SO_4;$$

with the potassium salts:

$$Cl < \text{acetate} < \text{citrate}$$

and

$$Br < I < CNS < NO_3,$$

the ion least effective in preventing swelling being given first.

Equimolar concentrations of the chlorides inhibited swelling in the series:

$$NH_4 < Na < K < Sr < Ba < Mg < Cu$$

Höber's microscopic studies (9) indicate that different ions have a specific effect on the central nervous system tissue. When the sciatic nerve of the frog is immersed in equimolar solutions of different salts for twenty-four hours, both the axis cylinder and medullary sheath present a different microscopic picture. Taking depth of color (the nerve was stained with toluidine blue) and swollen appearance as his criteria, Höber arranged the anions in the series:

$$\text{tartrate}, SO_4, \text{acetate}, Cl$$

and the cations

$$Na, Li, NH_4, K$$

The conductivity of the nerve he correlated with its colloidal consistency.

The differential water absorption by brain tissue we have attributed to differences in physico-chemical constitution. It should be of both speculative and practical interest to know whether the ions of the Hofmeister series exercise a differential effect on the hydration of the several portions of the brain substance. Dokan's experiments (10) with konyaku-mannane, obtained from the foodstuff konyaku of Japan, confirmed the advisability of the present investigation. A comparison of the colloidal behavior of agar with that of konyaku shows that specific ions have widely different effects on these two substances. The effect of electrolytes on agar is only to diminish the hydration while on konyaku some diminish and others increase the water absorption as compared with the swelling in distilled water. Furthermore the serial arrangement of the ion effects on the two substances is different.

EXPERIMENTAL METHODS. A rabbit was decapitated, the brain removed immediately and divided into four parts—cerebral hemispheres, cerebellum, mid-brain and medulla—in the manner described in a previous paper (11). These four parts were divided into symmetrical halves and each half-portion was dropped into a weighing bottle containing 15 cc.

of one salt solution, its corresponding half into another bottle containing the same amount of a different salt solution. Tenth molar solutions were used throughout the experiment, potassium salts for a comparison of the anion effects and chlorides for the cation. The weight of the brain portion was determined by subtracting the weight of the bottle plus solution from the weight of the bottle plus solution plus brain substance. At specified intervals each portion of the brain was placed on a watch crystal, weighed and returned immediately to the salt solution. Calculations were made of the percentage increase in weight.

The rate of water absorption in 0.1 M potassium sulfate was first determined for the anion series against distilled water as a control. In the cation series lithium chloride was used against distilled water. The effects on hydration of the brain by the different salts of the same series were then studied. The parts taken from one-half of the brain were immersed in K_2SO_4, those from the other half in KNO_3 or in KI; in the cation series LiCl was used for one-half of the brain, NaCl for the other or NaCl against $CaCl_2$, etc., etc.

Curves were plotted from absolute values, time in hours on the abscissa and percentage increase on the ordinate. Where the difference in the slope of the curves was a marked one, as with the sulfate, nitrate and tartrate curves, a direct comparative experiment with each ion against every other ion was deemed unnecessary. In this instance the sulfate was run against the tartrate in one experiment, and in another the sulfate against the nitrate; but not the tartrate against the nitrate. When, however, there was only a slight difference in the slope of the hydration curves as with the nitrate, chloride and iodide, several comparative studies were made, the nitrate against the chloride, the chloride against the iodide and the iodide against the nitrate.

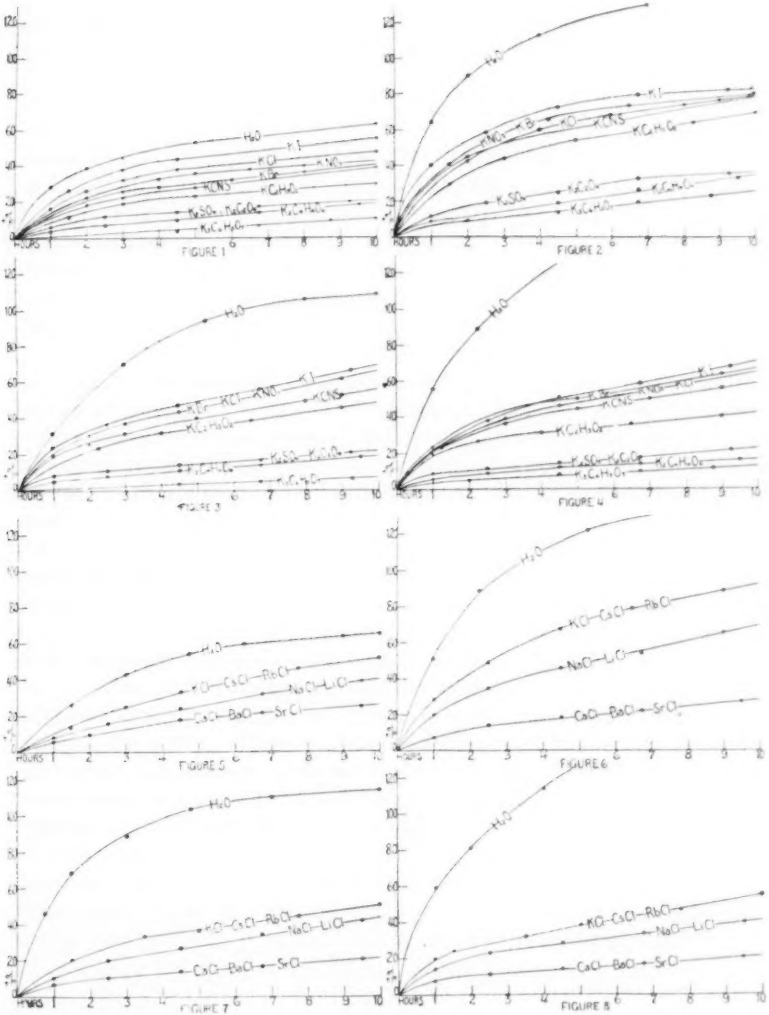
RESULTS. The graphs of figures 1 to 8 which are plotted from our experimental data show that the cerebral hemispheres, cerebellum, midbrain and medulla swell less in salt solutions than in distilled water. A difference in the effectiveness of the various ions in inhibiting swelling is evident from a comparison of the hydration curves obtained with equimolar solutions of different salts.

The anions inhibit the swelling in the four parts of the brain in the order:

$$\text{citrate} > \text{tartrate} > \text{oxalate}, SO_4 > \text{acetate} > CNS > Br > NO_3 > Cl > I,$$

the least amount of swelling occurring in the citrate solution, the greatest in the iodide.

The anions can be divided into two groups according to their inhibitory effect on swelling: citrate, tartrate, oxalate and sulfate comprising one group, thiocyanate, nitrate and the halogens the other. With the medulla the acetate occupies a position midway between the two groups.



Figs. 1 and 5. Absorption curve of cerebral hemispheres
Figs. 2 and 6. Absorption curve of cerebellum
Figs. 3 and 7. Absorption curve of midbrain
Figs. 4 and 8. Absorption curve of medulla

The amount of swelling of the cerebellum and medulla is the same in the bromide and nitrate, while the mid-brain swells equally in chloride, bromide and nitrate.

In the cation series some of the ions are equally effective in inhibiting swelling. The series can be arranged in the following order, the ion least effective being given first:

$$Ca, Ba, Sr > Na, Li > K, Cs, Rb$$

A comparison of the curves in figures 1 to 8 shows that the absolute amount of swelling of the four parts of the brain is different in the same solution. The water absorption of the medulla, however, is very close to that of the mid-brain. Relative to the hydration in distilled water, an ion has not the same inhibitory effect on the swelling of the several parts of the brain. Thus the mid-brain swells less than the cerebral hemispheres in potassium citrate, although it swells considerably more in distilled water.

Discussion. In a previous paper a colloid chemical hypothesis was proposed for a solution of the problem of mental disorders (11). The large amount of water contained in the brain and the coma associated with edema of that organ suggest that the functional integrity of mental processes might be related to the maintenance of a "normal" water content of the brain cells. The ions present in both the brain cells and the blood are unquestionably a factor in the physico-chemical equilibrium that exists between these two systems. Swelling or shrinking of the brain cells might be expected when the equilibrium is disturbed by a change in the concentration of ions.

If these experiments are representative of what occurs *in vivo*, it is evident that the several portions of the brain are affected differently by the various ions. Furthermore if our assumption is correct (11) that smaller groups of cells manifest specific hydration capacities which are affected differently, relative to one another, by the same chemical substance, our proposed colloid chemical basis of mental disorders becomes more probable. It is not difficult then to find for the innumerable forms of psychic disorders a possible explanation in the disturbance of brain protoplasm—blood equilibrium by variations in the concentration of ions and consequent effects on the hydration of the brain cells. We are aware that recent researches demand a modification of the doctrine of cerebral localization which has been widely accepted for the last fifty years; nevertheless one may feel justified in assuming that there exists some kind of correlation between psychic processes and localized areas of brain protoplasm.

It has been demonstrated by Weed and McKibben (12) that in animals a decrease in the size of the brain follows upon an intravenous injection of hypertonic salt solution. Cushing and Foley (13) and Sachs and Belcher

(14), applying these results to patients, found that, after an intravenous injection of sodium chloride, swollen brains decreased in size and cerebral operations were thereby rendered less difficult. Barach and his co-workers (15) administered hypertonic salt solutions intravenously to six patients with brain tumor and to one patient with a fractured skull. The immediate effect was a marked increase in plasma volume, between 15 and 30 per cent of the original blood volume, and a lowering of cerebrospinal pressure. Symptomatic improvement occurred in all the patients, manifested chiefly in the relief of headache and stupor. These observations indicated that the volume change of the blood was due, in part at least, to an egress of fluid from the brain. Fay (16) has shown that different salts are not equally effective in dehydrating the brain. Magnesium sulfate is almost twice as effective as sodium chloride.

The recent experiments of Elsberg and Pike (17) have a bearing on the present discussion. These investigators found that the susceptibility of cats to convulsive seizures which are produced by absinth is increased whenever the intracranial pressure is raised by an intravenous injection of distilled water. On the other hand it is diminished when intracranial pressure is lowered by the intravenous injection of hypertonic salt solutions. The authors were unable to determine whether the susceptibility of the animal to absinth is due to the increase or decrease of intracranial pressure itself or whether it occurs secondarily through an effect upon circulation of the blood in the motor areas. Nevertheless it is certain that variations in intracranial pressure have a decided effect. It is suggested that the benefit of the starvation treatment in some cases of epilepsy might be due to a lowering of intracranial pressure. A report is forthcoming from these investigators upon the results of experiments in which an increase of pressure over localized areas of the brain was produced. The unilateral convulsive seizures of the so-called Jacksonian epilepsy, they believe, may be due to a considerable degree to localized variations in intracranial pressure. These experiments together with our own and the clinical observations to which we have referred lend support to our colloid chemical explanation of mental disorders.

SUMMARY

1. The cerebral hemispheres, cerebellum, mid-brain and medulla of the rabbit's brain swell less in salt solutions than in distilled water.

2. Different ions have a different inhibitory effect on the swelling of brain tissue.

3. The anions of the potassium salts inhibit water absorption by the four parts of the brain in the order

citrate > tartrate > oxalate, SO_4 > acetate > CNS > Br > NO_3 > Cl > I

The series reads: the least amount of swelling in citrate, the greatest in iodide.

4. The difference in the inhibitory effect of nitrate and the halogens is almost negligible. Swelling of the mid-brain in chloride, bromide and nitrate is the same. Swelling of the cerebellum and the medulla is the same in bromide and nitrate.

5. In the cation series the bivalent ions are most effective in inhibiting swelling. Sodium and lithium on the one hand and potassium, caesium and rubidium on the other, have the same dehydrating effect. That of the former group however is greater than that of the latter.

6. The cation series of the chlorides follows the order

$$Ca, Ba, Sr > Na, Li > K, Cs, Rb$$

7. The absolute amount of water absorption of the four parts of the brain, determined by percentage increase in weight, is different in the same salt solution.

8. Relative to the swelling of the brain portions in distilled water, an ion has not the same inhibitory effect on the several parts of the brain.

9. Further remarks are added to our previous discussion (11) of a possible colloid chemical basis of mental disorders.

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DIURNAL VARIATIONS IN BLOOD SPECIFIC GRAVITY IN NORMAL DOGS AND HUMANS

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In the summer of 1925 we undertook to obtain physiological standards for blood specific gravity according to the falling drop method devised by Barbour and Hamilton (1). Dogs and humans were studied. The subjects rested quietly in the laboratory from seven in the morning till ten at night with very light exercise in connection with eating. Blood was drawn at intervals varying from one to two hours during this period, and the subjects were again observed on the following morning.

The blood was drawn from the femoral artery in the case of the dogs by means of a clean dry syringe, and transferred at once to the measuring pipette. In the human subjects, two lancings were made close together, either on finger tips or ear lobes, and if the skin had just previously been rubbed with hot water and dried, sufficient free flowing blood for the measured drop could be secured without difficulty. The observed specific gravities in each case were referred to water at the temperature at which the determinations were made. This was usually between 20°C. and 22°C.

The falling drop method for the determination of blood specific gravity is very exact (2). In a series of twenty-five consecutive and rapidly made determinations on the same sample of oxalated blood, we found the probable error of a reading to be $\pm$ 0.00012. As the originators of the method have shown, the probable error may be reduced as desired by increasing the falling time.

In table 1 may be found the detailed observations in a typical human subject. In table 2 are summarized the data obtained from the human subjects. Eleven series of observations were made on six normal male subjects between twenty and thirty years of age, while eight series of observations are recorded on seven healthy female subjects between seventeen and twenty-seven years of age. The highest and lowest blood specific gravity for the day is noted, and the maximum variation for the observation period in each case. The highest blood specific gravity figures obtained during the experiments for the different men ranged from 1.0560 to 1.0596, and averaged 1.0579. The figures for the low point in the diurnal period for the same individuals ranged from 1.0526 to 1.0561, and averaged

1.0545. In the case of the women the figures were lower, as consistently noted in reports based on other methods. The upper limits in the diurnal period varied in the different female subjects from 1.0517 to 1.0569, averaging 1.0548, while the corresponding low limits ranged from 1.0499 to 1.0541, and averaged 1.0521. It is interesting that the average maximum variation during the day was less for the women than for the men, being 0.0027 for the former and 0.0033 for the latter. This may be a reflection of the lighter meals taken by the women.

The general average of a total of 118 observations on the six normal males is 1.0565. For the seven normal women, the grand average of a total of 92 observations is 1.0533. These averages are slightly lower than those quoted by other investigators using other methods.

TABLE 1

Diurnal variation in blood specific gravity and erythrocytes in subject M 5. Activity during the day consisted in reading and writing; walked about 600 feet for each meal

TIME	BLOOD SPECIFIC GRAVITY	ERYTHROCYTES	REMARKS
		millions per cmm.	
7:30 a.m.	1.0586	5.46	Light breakfast at 8:00 a.m.
9:00 a.m.	1.0573	5.32	
10:00 a.m.	1.0578	5.28	Drank 300 cc. water at 10:15 a.m.
11:00 a.m.	1.0561	5.30	
12:00 noon	1.0570	5.36	Light lunch at 12:30 p.m.
1:30 p.m.	1.0573	5.33	
2:30 p.m.	1.0563	5.17	
3:30 p.m.	1.0568	5.29	Drank 300 cc. water at 3:45 p.m.
4:30 p.m.	1.0559	5.12	
5:30 p.m.	1.0568	5.23	Moderate dinner at 6:00 p.m.
7:00 p.m.	1.0565	5.19	
8:00 p.m.	1.0553	5.28	
9:00 p.m.	1.0571	5.39	
10:00 p.m.	1.0568	5.22	
8:00 a.m.	1.0589	5.36	

In table 3 are summarized the findings in three male and three female dogs. Probably due to the wide differences in general condition and state of nutrition of these animals, a much wider variation in the diurnal range of the blood specific gravity was noted than in the case of humans. The difference between the sexes in this inadequate series was not significant. The average maximum diurnal variation was found to be 0.0044, which is considerably higher than for humans.

Comment. Our observations confirm, in general, the extraordinary study on 362 individuals made with a cumbersome method by Jones (3) many years ago. He found *a*, the average blood specific gravity for males in

TABLE 2

Summary of diurnal variations in blood specific gravity in normal human subjects giving maximum and minimum determinations for the day, together with the difference

SUBJECTS	BLOOD SPECIFIC GRAVITY		DIFFERENCE
	Maximum	Minimum	
M 1	1.0565	1.0549	0.0016
M 1	1.0562	1.0536	0.0026
M 1	1.0586	1.0547	0.0039
M 1	1.0560	1.0526	0.0034
M 2	1.0594	1.0566	0.0028
M 3	1.0596	1.0561	0.0035
M 4	1.0577	1.0531	0.0044
M 5	1.0589	1.0553	0.0036
M 5	1.0592	1.0556	0.0036
M 5	1.0578	1.0548	0.0030
M 6	1.0579	1.0539	0.0040
Average maximum variation males			0.0033
F 1	1.0567	1.0538	0.0029
F 1	1.0536	1.0502	0.0034
F 2	1.0551	1.0518	0.0033
F 3	1.0569	1.0532	0.0037
F 4	1.0523	1.0499	0.0024
F 5	1.0558	1.0541	0.0017
F 6	1.0517	1.0499	0.0018
F 7	1.0566	1.0539	0.0027
Average maximum variation females			0.0027

TABLE 3

Summary of diurnal variations in blood specific gravity in dogs

SEX	WEIGHT	BLOOD SPECIFIC GRAVITY		DIFFERENCE
		Maximum	Minimum	
	kgm.			
M	15	1.0492	1.0468	0.0024
M	18	1.0644	1.0576	0.0068
M	12	1.0532	1.0501	0.0031
F	14	1.0498	1.0453	0.0045
F	11	1.0577	1.0515	0.0062
F	16	1.0547	1.0509	0.0036
Average maximum variation				0.0044

our age group to be 1.0570 and for females 1.0540; *b*, the ingestion of an ordinary mixed meal is followed usually by a fall in the blood specific

gravity; *c*, the highest blood specific gravity is found just before breakfast and the lowest at night after dinner; *d*, gentle exercise is followed by a fall in blood specific gravity, but moderate or severe exercise results in a rise, and *e*, the blood specific gravity varies considerably in different persons. To this last observation, we may add that it also varies considerably from day to day in the same individual, as shown in our subjects M 1, M 5 and F 1.

We desire to call attention to the need of including normal diurnal variations more frequently in physiological standards, particularly in those referring to the blood. Variations from conventional norms are significant only when they are well outside the limits of the average diurnal changes as determined by repeated observation in a sufficiently large number of healthy individuals going through a light daily routine, or at complete rest. The importance of such determinations with regard to the erythrocytes and hemoglobin was shown long ago in a neglected study by Reinert (4); and has also been emphasized by Ward (5), Dreyer, Bazett and Pierce (6), Rabinovitch (7) and Osgood (8). In the case of the leukocytes, the surprising extent of the diurnal variation has been demonstrated by Sabin, Cunningham, Doan and Kindwell (9).

SUMMARY

With the falling drop method of Barbour and Hamilton for the determination of blood specific gravity, the average maximum diurnal variation in six dogs was found to be 0.0044. In normal men it was 0.0033, and in normal women 0.0027. The general average of 118 observations of the blood specific gravity in six normal men was 1.0565, while in 92 observations on seven normal women it was 1.0533.

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THE GLYCOGEN CONTENT OF FROG'S MUSCLE AFTER INJECTION OF INSULIN AND ITS RELATION TO CONTRACTION

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The importance of glycogen in muscular contraction is well known through the work of Meyerhof and others. The question arises, can muscle contract in the absence of glycogen? Chaikoff (1925) suggests that if rabbits survive a convulsive insulin fit "there must be glycogen present in the muscles. Death is due to the absence or almost complete absence of glycogen in the muscles." The frog was thought to be a favorable animal upon which to try out this hypothesis.

Pflüger's method of estimating glycogen was used in these experiments, glycogen being determined as glucose. Because of the rapid changes in glycogen content known to occur in mammalian muscle after removal from the body, great care was taken to get the frog's muscle into KOH as soon as possible after experimental treatment. This was accomplished in less than 20 seconds, the weight of the muscle being obtained by the difference in weight of the KOH container before and after addition of the muscle.

Lovatt Evans (1926) has questioned the accuracy of the determination of glycogen in small amounts by the ordinary methods. In a series of control estimations using known quantities of glycogen of the same order as those found in our experiments, and also using like quantities of reagents as in our experiments, the following checks were obtained: 23 mgm. recovered out of 24.5; 12.6 out of 12.4; 1.3 out of 1.2; 0.45 out of 0.5. These results are the average of several determinations and the departure from the average may be as much as 75 per cent in amounts less than 1 mgm. Indication of the presence of glycogen was shown in amounts from 0.5 to 0.2 mgm. but the amount recovered might vary from half to twice the original amount. Usually 0.1 mgm. titrated the same as the blank. Therefore if in our experiments there is a report of no glycogen this signifies that if glycogen is present the amount is less than 0.2 mgm. per gram of muscle.

In the first series of experiments excised gastrocnemii were used. One muscle from a frog was placed at once in KOH and the other was arranged for stimulation and recording. The stimuli were maximal shocks from an

inductorium sent in at the rate of 2 per second. The muscle was afterloaded and lifted a weight of 10 grams. The results of five experiments on normal frogs gave an average of 22.8 mgm. glycogen per gram tissue in the unstimulated muscles and 20.1 in muscles so fatigued that they would no longer respond to stimulation. In frogs injected with insulin and kept at room temperature there is little effect on the glycogen content of the muscles until after 24 hours when convulsions begin; in one case 21.9 mgm. glycogen, the normal amount, were found 10 hours after insulin; and in another 23 hours after insulin, 22.2 mgm. glycogen were found. But during and after convulsions as low as 4.7 mgm. were found even in unstimulated muscles. The results obtained on fourteen frogs, each injected with 5 units of insulin 24 hours or more previously, gave an average of 10.5 mgm. glycogen per gram tissue in the unstimulated muscle and 9.8 in the fatigued muscle. In no case was the glycogen content less than 4.5 mgm.

TABLE 1

Normal frogs

GLYCOGEN IN FATIGUED MUSCLE	LENGTH OF STIMULATION	GLYCOGEN IN UNSTIMULATED MUSCLE	DIFFERENCE	GLYCOGEN IN LIVER
	hours			
11.8	2	17.0	5.2	83.4
3.9	2	16.5	12.6	81.5
8.4	1¼	21.0	12.6	107.7
5.0	½	26.5	21.5	
11.1	½	17.4	6.3	
10.9*	½	21.6	10.7	
6.3*	½	16.7	10.4	
6.1†	½	19.8	13.7	
7.5†	½	12.8	5.3	
11.6†	½	14.3	2.7	
14.3†	½	19.6	5.3	
17.2†	½	21.0	3.8	
15.3‡	½	18.2	2.9	82.0
11.0‡	½	15.5	4.5	136.0

* Skin and muscle cut as if to remove liver, but liver left intact.

† Liver removed 24 hours previously.

‡ Strychnine injected.

In all these experiments fatigue occurred in about 20 minutes. It was thought that if a muscle with intact circulation were used a fatigue-level might be reached, and through continued contraction all the store of glycogen might be used up. In experiments to test this point the frogs were tied to a board and a bent pin carefully inserted into the tendo-Achilles for connection with a writing lever. One electrode was attached to the

pin, the other to a fine wire passed loosely around the knee. The whole arrangement was adjusted so as not to interfere with circulation. It was found that even after 5 hours' stimulation at the rate of 2 shocks per second, muscles still responded, making an excursion of a centimeter or more on the smoked paper.

The results on frogs without insulin are given in table 1. The average amount of glycogen in the unstimulated muscle was 18.4 mgm. and in the stimulated muscle 10.0, an average difference of over 8 mgm. It was thought that by removing the liver the chief supply of glycogen would be

TABLE 2

Frogs injected with 5 units of insulin

TIME AFTER INJECTION	GLYCOGEN IN FATIGUED MUSCLE	GLYCOGEN IN UNSTIMULATED MUSCLE	DIFFERENCE	GLYCOGEN IN LIVER
hours				
24*†	7 8	13 2	5 4	
24*‡	9 0	14 6	5 6	
24*	5 5	8 0	2 5	
32	6 4	16 5	10.1	114
36	4 1	7 0	2 9	74 2
30	10 9	16 2	5 3	
30	0	6 3	6 3	
30	9 7	22 3	11 6	
30	0	2 3	2 3	
40	0	3 3	3 3	56 1
40	1 6	1 9	0 3	82 0
40	2 6	8 2	5 6	84 0
40	3 2	6 4	3 2	
40	4 4	10 3	5 9	55 3
40	0	2 7	2 7	56 3
44	1 9	6 8	4 9	

* No convulsions.

† Liver removed.

‡ Liver removed and strychnine injected.

lacking, and the muscles, especially after continued stimulation, would show a low glycogen content. This was not the case, as seen in the table. Injection with strychnine causing vigorous convulsions also did not avail to lower the glycogen content of muscles. Frogs injected with 5 to 10 units of insulin usually continue to have convulsions at intervals throughout the second day after injection, and on subsequent days until death. The glycogen content even of unfatigued muscles in these frogs is quite low, especially if convulsions have been violent and prolonged (table 2). The average amount of glycogen in the unstimulated muscle was 9.1 mgm. and in the fatigued muscle 4.2, an average difference of nearly 5 mgm.

In four cases no glycogen was detected in muscles which had been fatigued for 30 minutes, nevertheless the height of contraction registered on the drum by these glycogen-free muscles was no different from that registered by normal frog's muscle under similar conditions (figs. 1, 2, 3).

To test whether glycogen would be restored in the fatigued muscle, the following experiments were undertaken. It was found that the glycogen content of the two gastrocnemii of a normal resting frog is approximately the same, e.g., in one individual (a spring frog)[1] the right gastrocnemius contained 7.9 mgm. glycogen per gram muscle and the left 8.2. Therefore it is possible to use one leg as a check on the other. Two methods

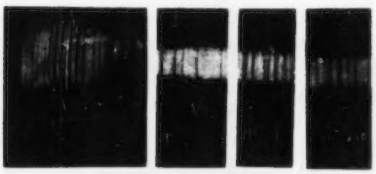

Fig. 1.

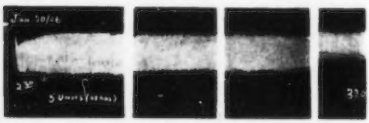

Fig. 2.

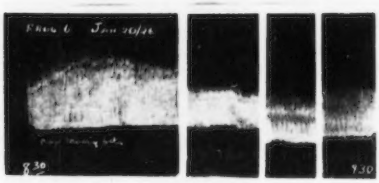

Fig. 3.

Fig. 1. Record of stimulated gastrocnemius of normal frog. Glycogen content 18 mgm. per gram after stimulation.

Fig. 2. Record of stimulated gastrocnemius of insulin frog. No glycogen found.

Fig. 3. Record of stimulated gastrocnemius of insulin frog at time of convulsions.
No glycogen found.

of procedure were adopted. *a*. The frogs were tied to the board in the usual manner and one leg stimulated for 1 hour, after which it was allowed to rest for a given time. Then the other leg was stimulated for 1 hour,

[1] There was a considerable difference in the glycogen content of muscles of the laboratory-kept winter frogs used in the first part of this work, and in the newly caught spring frogs used in the later experiments. The latter had just appeared after hibernation in a marsh and were swimming about vigorously in the activities of the breeding season. The winter frogs had an average of 19 mgm. glycogen per gram tissue, the spring frogs less than half this amount, e.g., 7.9, 8.0, 6.8 etc.

and at the end of this time both gastrocnemii were removed and dropped into KOH. *b.* Both gastrocnemii were stimulated at the same time; one leg was then tied with a mass ligature just above the knee and the gastrocnemius on that side removed and placed in KOH while the other was allowed to rest. The first method gave better results.

TABLE 3

	GLYCOGEN IN FATIGUED AND RESTED MUSCLE	LENGTH OF REST	GLYCOGEN IN FATIGUED MUSCLE	GAIN	GLYCOGEN IN LIVER
		hours			
Winter frogs	19.5	2½	11.8	7.7	85.0
Spring frogs	4.9	4	2.3	2.6	61.0
	8.7	3	6.9	1.8	
	4.4	3	3.0	1.4	38.0

TABLE 4

Frogs injected with insulin

	HOURS AFTER INSULIN	GLYCOGEN IN FATIGUED AND RESTED MUSCLE	LENGTH OF REST	GLYCOGEN IN FATIGUED MUSCLE	GAIN	GLYCOGEN IN UNSTIMULATED THIGH MUSCLES	GLYCOGEN IN LIVER
			hours				
Winter frogs	48	0.1	1	0.3	0.2		32
	48	0.3	3	0.3	0		138
	48	0.2	2	0.4	0.2	13.5	131
	48	0	3	0	0	11.4	115
	48	0	2½	0	0	11.6	70
	48	0	2	0	0	5.9	66
	44	5.0	4	1.9	3.1		
Spring frogs	48	0.9	3	0.7	0.2	8.0	45.4
	48	6.5	4	1.2	5.3		
	48	3.6	3	2.3	1.3		99
	48	4.1	5½	2.6	1.5		26.8
	44	2.1	3			6.8*	

* Unfatigued gastrocnemius.

Table 3 gives the results on frogs without insulin and table 4 those for frogs injected with insulin. It will be seen that there is recovery if the glycogen content is not too low. In three of the rested muscles no glycogen was detected and in three others practically none. The same was true of the fatigued muscles of these frogs. When the frogs of this lot were put on the frogboard for stimulation they had had convulsions at frequent intervals for 24 hours. Insulin renders the frogs hypersensitive and moving them about with a glass rod will often induce convulsions. Also rais-

ing their temperature increases the tendency to convulsions. Both these means had been employed throughout the second day after injection of insulin, so that when they were put on the board most of them were unable to sit up, but they still gave the wink reflex. The contractions resulting from electrical stimulation, even after an hour, were undistinguishable from those of normal frogs. It is evident, therefore, that muscular contraction can take place with practically no glycogen present.

It is extremely difficult to prove a negative and these experiments by no means prove that contraction can take place in *absence* of glycogen, they show that contraction can take place in the presence of so small an amount of glycogen that it is not detectable by the method used, i.e., less than 0.2 mgm. per gram tissue. It would seem that the chain of reactions involved in the change, glycogen $\rightleftarrows$ lactic acid, can go on even if the tendency of the reaction to go to the right is so great that no glycogen can be detected by the usual chemical means. Studies are now in progress to determine the lactic acid content of muscles under these same conditions.

CONCLUSIONS

The muscles of frogs can be rendered practically free from glycogen by causing them to have prolonged and violent insulin convulsions, i.e., the glycogen content is less than 0.2 mgm. per gram of muscle, the limit of detectability by the method of analysis used (Pflüger's). Yet under these conditions the muscle is able to contract for hours upon being stimulated in the intact animal with maximal shocks from an inductorium, the height of contractions being virtually the same as in the normal frog's muscle stimulated for the same length of time.

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POSTERIOR PITUITARY EXTRACT AND CHOLESTEROL METABOLISM

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The evident relationship existing between the pituitary gland and fat metabolism has made it advisable to study the effect of posterior pituitary extract on blood cholesterol.

There is already theoretical data (1), (2) regarding this question and in the present article experimental results are set forth.

Twenty young rabbits weighing approximately 2 kgm. were used. The blood for the cholesterol estimations was withdrawn in the afternoon after a six-hour fast. We found however that the cholesterol was not altered to any appreciable degree by ordinary feedings, but in order to obviate error the six-hour fast was used. Blood cholesterol estimations were made by Sackett's modification of Bloor's method (3). Each rabbit received subcutaneously 1 cc. of pituitary extract (Burroughs Wellcome & Co.) for ten days. The rabbits received their usual diet of oats, hay and water, varying in no way from the diet of the other rabbits.

Estimations were again made after a six-hour fast on the eleventh day.

The results obtained were those given in protocol (see page 650).

The data at hand show that there was a 30 per cent increase in blood cholesterol in 85 per cent of the rabbits and a 14 per cent decrease in 10 per cent, while no change was present in 5 per cent.

This protocol shows that under the conditions of the experiment there was an average increase of blood cholesterol amounting to 30.87 per cent, taking the group as a whole. Eighty-five per cent showed an increase averaging 37.93 per cent of the fasting pre-treatment level. There was no change in one rabbit (5 per cent) and a decrease of 7.33 and 20.2 respectively in two (15 per cent) of the animals. The greatest increase found was in rabbit 4 (131 per cent) and the smallest increase in this group was in rabbit 6 (10.01 per cent). The average normal fasting level was 158 mgm. per 100 cc. of blood and the average post-treatment level 198 mgm.

THEORETICAL CONSIDERATIONS. From a clinical standpoint it seems reasonable to assume that the pituitary gland influences cholesterol metab-

RABBIT	FASTING CHOLESTEROL BEFORE TREATMENT	FASTING CHOLESTEROL AFTER 10 DAYS' TREATMENT	PER CENT OF CHANGE PLUS OR MINUS
	mgm. per 100 cc.	*mgm. per 100 cc.*	
I	188	235	+25.0
II	120	160	+33.3
III	217	173	−20.2
IV	115	266	+131.0
V	143	235	+64.25
VI	218	240	+10.01
VII	114	180	+58.0
VIII	104	160	+53.8
IX	171	228	+33.6
X	160	229	+43.1
XI	150	208	+38.65
XII	160	180	+12.50
XIII	184	171	−7.33
XIV	150	150	0
XV	145	192	+32.4
XVI	150	192	+28.0
XVII	150	173	+15.23
XVIII	171	218	+27.50
XIX	150	192	+28.0
XX	160	177	+10.6

olism. Hypopituitary states result in increased fat storage due to the fact that the stimulus to fat metabolism is lessened by the lessened pituitary secretion. Increased pituitary secretion results in the tall, slender type of individual, whose fat is burned up through hypersecretion. Experimentally produced hypopituitarism results in obese animals.

Warthin (4), in studying two cases of pituitary dystrophy, found that the cholesterol infiltration or retention occurs similarly to that obtained by overfeeding with cholesterol and to the lipoidosis of diabetes and of Gaucher's disease.

Goñalons (1) found that the pituitary gland contains a considerable amount of cholesterol, chiefly in the posterior lobe. In dogs an increase in circulatory cholesterol results from hypophysectomy. He believes that the cholesterol in the pituitary is secreted into the blood.

A study of the selective action of the pituitary gland upon mesenchymal tissue lends additional support to the opinion that the pituitary secretion influences cholesterol metabolism.

In previous communications (5) data were presented which demonstrates this control which the pituitary exercises over mesenchymal tissues. While it is not pertinent to the present article to discuss all these tissues, nevertheless the evident relationship of the pituitary to bone and cartilage is pointed out. The pharmocological effect of subcutaneous injections of

posterior pituitary extract is, however, pertinent to this subject. It causes contraction of the blood-vessels, contraction of smooth muscle, such as that of the uterus, bladder, etc., and a varying effect on the renal cells, opinion being divided as to whether the latter is diuretic or antidiuretic.

The two mesenchymal tissues that we are concerned with at present are the fat cells and the suprarenal cortex and the relationship of the pituitary to fat metabolism has been previously cited.

The rôle of the suprarenal cortex in cholesterol metabolism is well recognized, but the mechanism by which it influences the metabolism is not yet discovered.

It was stated in another communication (5 d) that hypo- and hyperplasia of the pituitary gland were accompanied by hypo- and hyperplasia respectively of the suprarenal cortex. Zander (6) noted in studying forty-three anencephalic fetuses that the suprarenals were hypoplastic only in defects of the anterior portion of the brain which includes the pituitary gland, and therefore accounts for the suprarenal cortex defect. Kohn (7) likewise believes that suprarenal hypoplasia is secondary to changes in the pituitary in anencephalic fetuses. Browne (8), studying five anencephalic fetuses, found absolutely no trace of a pituitary gland and these fetuses showed marked suprarenal hypoplasia.

Attention is called to the fact that the reticulo-endothelial system is derived from the mesenchyme and this system is likewise involved in cholesterol metabolism.

In another paper we will discuss the practical clinical application of the posterior pituitary gland relationship to cholesterol metabolism and the mesenchymal tissues.

CONCLUSIONS

1. Posterior pituitary extract increases the blood cholesterol in rabbits if injected over a course of ten days.

2. Explanation for its action can be found in the selective action of pituitary secretion on mesenchymal tissues.

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THE SEGMENTAL INNERVATION OF SKELETAL MUSCLES OF THE FROG[1]

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Gad (1882) was the first to examine the plurisegmental innervation of the gastrocnemius of the frog. He found that the tension caused by simultaneous stimulation of the two nerves was the same as the sum of the tensions after stimulation of the two nerves separately. The result was the same whether he used single induction shocks or stimulated tetanically. Cattell and Stiles (1924) found that the tension produced after stimulation of one nerve alone was about the same as the tension produced by stimulation of the two nerves. A number of later investigators, Samojloff (1924), Fulton (1925), de Boer (1926), on the other hand, found that the tension after stimulation of the two nerves was always smaller than the sum of the tensions caused by stimulation of each nerve separately.

Cattell and Stiles also found that fatigue produced by rhythmical stimulation of one anterior root did not affect the normal response obtained by stimulating the other anterior root. They explain this by assuming that stimulation causes fatigue on the end-plates of the nerves. These investigators thought that the greater part of the muscle fibres of the gastrocnemius were innervated from two segments. It must indeed be remembered that the possibility of fatigue of the nerve endings is not only excluded, but is just as probable as fatigue of the contractile tissue. In any case experiments with isometric registration and also those during which fatigue is induced are inadequate to give a complete answer to the question.

I therefore took another course (1925). I allowed the circulation of the blood to continue, after having by means of a pin made a cross lesion of the spinal marrow, a little below the skull. Then the abdomen was opened and a ligature tied around the 8th and 9th nerves (numeration of Langley and Orbeli, 1910), close to the spinal column. These nerves were cut near the exit from the vertebrae and put on separate stimulation electrodes. The thigh was firmly pinned down and the tendon of the gastrocnemius muscle connected with a recording lever. Then 5 to 10 drops of acetas

[1] Reported at the 12th International Physiological Congress in Stockholm, August, 1926.

veratrini (1 per cent) were injected subcutaneously. As soon as an induction shock of one of the nerves caused a veratrine curve the experiment was begun. As is well known, the veratrine effect disappears when a stimulus is repeated a few times. Therefore one nerve was stimulated at intervals of two seconds till the veratrine effect had disappeared; then the other nerve received an induction shock the effect of which always was a perfect veratrine curve. The same result was also obtained when after 3 or 4 induction shocks at intervals of five or more seconds the veratrine effect had disappeared. In this experiment the result can scarcely be ascribed to fatigue of the end-plates, for 3 or 4 induction shocks distributed over twenty or more seconds cause no fatigue. Besides, in my first communication (1925), I mentioned a control experiment that also excludes the fatigue of the end-plates. Here I rhythmically stimulated the sciatic nerve of a frog, poisoned with veratrine till a single twitch of the gastrocnemius resulted. When afterwards the muscle was directly stimulated, there never resulted a veratrine curve, but always a single twitch. When through indirect stimulation the veratrine effect has disappeared it can never be brought about again by direct stimulation. This shows that fatigue of the end-plates cannot account for the disappearance of the veratrine effect after indirect stimulation. Katz (1926) expresses the opinion that it would be possible to defend the idea that veratrine acts on the nerve endings. According to him the veratrine effect would then disappear in consequence of the rhythmic indirect stimulation through fatigue of the nerve endings. All the arguments against fatigue of the end-plates mentioned above, however, are opposed to this conception. Besides an experiment which has been known for the last 20 years or more disproves it; i.e., it is possible to get real veratrine curves by direct stimulation of the muscle after poisoning with veratrine and curari, which destroys the connection between the end-plates and the muscles. Hence I adhere to the conclusion that it has been proved by my veratrine experiments that in the gastrocnemius there are two sets of muscle fibres, each of which are innervated from one anterior root.

In a second communication (1926) I showed that the action current curve registered by monophasic leads upon stimulation of both nerves simultaneously is equal to the sum of the monophasic action current curves which result from stimulation of the two nerves separately. This justified the conclusion that the muscle fibres of the gastrocnemius are all, or almost all, monosegmentically innervated. This conception is supported by the experiments of Katz (1926) which proved that the quantity of heat produced after stimulation of the two nerves simultaneously is equal to the sum of the quantities produced after stimulation of the two nerves separately. Samojloff and Wassiljewa (1925) come to the same conclusion, as does also Quednau (1926).

Samojloff (1924) who used excised, bloodless nerve-muscle preparations for his experiments, stimulated one nerve rhythmically, till fatigue ensued. Then stimulation of the other nerve caused twitches without signs of fatigue. If this second nerve was again rhythmically stimulated till fatigue had arisen, he saw afterwards very slight recovery after stimulation of the first. As this recovery was so very slight (see fig. 3, p. 700, Pflüger's Archiv, cciv, 1924) I repeated these experiments on frogs with intact circulation, and on the contrary got a very distinct recovery (see fig. 3, p. 218, Journal of Physiology, lx, 1925). This result indicates that the two sets of muscle fibres work independently of each other in a chemical and electrical sense. This means that the metabolic products formed in the contracting fibres do not readily diffuse to the inactive fibres since the latter yielded curves without signs of fatigue. Besides, if this chemical insulation were absent the before mentioned recovery would not have been so pronounced.

Electrically the two groups function apart also. This means that the action currents which accompany the contraction of one set of fibres do not influence the other set. Here I have in mind the well-known experiment of the secondary twitch of Matteucci. In this experiment the action currents of one contracting muscle stimulate the nerve of a second nerve muscle preparation.

Thus being convinced of the monosegmental innervation of the muscle fibres of the gastrocnemius I asked myself where the one and where the other set lies in the muscle. Are the fibres of the two sets mixed up or lying in distinct groups? To answer this question I made a nerve muscle preparation (sciatic nerve and gastrocnemius) and suspended the muscle from the fixed femur (1925). Then I stimulated the 8th nerve, whereupon the gastrocnemius curved more to the ventral side (i.e., the upper side of the muscle, when the frog lies on its back). If the 9th nerve was then tetanically stimulated, the muscle stretched in a more vertical direction, or it pointed to the dorsal side. From this observation I inferred that the group of muscle fibres which is innervated by the 8th nerve lay along the ventral side of the muscle, whereas the group of fibres innervated by the 9th nerve lay along the dorsal side.

To further test this inference I carefully separated ventral and dorsal strips of the muscle beginning at the tendonous end. Each strip was connected with a recording lever and the whole preparation so fixed that the contractions of the two strips could not influence one another, while the contraction of the remainder could not distort the contraction curves of the strips. Under these conditions I found that the dorsal strip responded only to stimulation of the 9th nerve, while the ventral strip responded only to stimulation of the 8th nerve. Thus it was possible to isolate groups of muscle fibres corresponding to specified segmental innervation.

The situation of the two parts of the segment[2] in the gastrocnemius is not constant. Sometimes the 8th myotome extends more, sometimes less in the dorsal direction; likewise the 9th myotome may extend more or less towards the ventral side. Consequently the curve towards the ventral side, upon stimulation of the 8th spinal nerve, is at times more strongly pronounced than at other times. Likewise the muscle can, after stimulation of the 9th spinal nerve, show all the changes in form between a ventral and a dorsal direction.

Occasionally the 7th spinal nerve also innervates the gastrocnemius. In such cases stimulation of this nerve also causes a curving of the muscle to the ventral side and the 7th myotome takes the place of the 8th. The 8th myotome extends then in a more dorsal direction and the 9th lies along the dorsal side of the muscle. Sometimes the 10th spinal nerve also takes part in the innervation. This part of the 10th myotome then extends along the dorsal side of the muscle. The 9th then lies in a more ventral direction and the 8th extends along the ventral side. In this way the serial arrangement is preserved for the parts of the myotome. As regards the dermatomes it has long been known (Bolk and Sherrington) that the extremities can develop at different heights with respect to the spinal marrow; with regard to the skeletal muscles nothing has heretofore been known. If an extremity develops more caudally or more cranially the myotomes shift in the same way as the dermatomes. It was also determined, by my method and double suspension, that the dorsal side of the tibialis anticus longus is innervated by the 9th spinal nerve and the ventral side by the 8th. I have now examined several muscles of the upper leg.

Method. The distal end of the muscle is loosened from its surroundings for about a third of its length. Then a lengthwise incision is made and the proximal end of each strip fastened on a cork plate so that the two strips, when contracting, could not influence one another, and in turn, could not be influenced by the unsplit part of the muscle. Each strip was connected to a lever. Then the 8th and 9th spinal nerves were alternately stimulated and the curves registered.

Results. These experiments were not so easily made on the muscles of the thigh as on the gastrocnemius. The cause is, probably, that the muscles of the thigh are thinner and are injured when loosened from their surroundings. The consequence was that more than once one of the two strips did not respond to nerve stimulation. Records of some of the successful experiments are given. Figure 1 is from the gracilis major after a lengthwise incision not far from the medial border. Thus the muscle was cut into a medial and a lateral strip; the medial record, *M*, is above and the lateral record, *L*, below. After tetanic stimulation of the 8th spinal nerve both

[2] The whole myotome is scattered over many muscles so that only a small part of it is in each muscle.

strips contract, but the contraction of the medial strip is the greater. After similar stimulation of the 9th spinal nerve only the lateral strip contracts. From this we conclude that the medial part of the muscle is only innervated by the 8th and the lateral part exclusively by the 9th nerve. Figure 2 is from the adductor magnus after an incision close along the lateral border. The lateral record, *L*, is above and the medial record, *M*, below. After tetanic stimulation of the 8th nerve only the medial strip contracts, while after stimulation of the 9th nerve both strips contract. The lateral strip was very narrow in this experiment so that the contraction curve is not large. Figure 3 is from the rectus femoris after an incision close along the dorsal border so that the muscle was divided into a dorsal and a ventral part. The ventral record, *V*, is above and the dorsal record, *D*, below. After stimulation of the 8th spinal nerve, only the ventral strip contracts and after stimulation of the 9th spinal nerve both strips contract. Here too the dorsal strip was very narrow.

By the same method the abductor magnus, the vastus externus and

Fig. 1

Fig. 2

Fig. 3

the semimembranosus were examined. In these muscles also the lateral (dorsal) border was innervated by the 9th nerve and the medial (ventral) border by the eighth. From these experiments we may conclude that of the various muscles of the thigh of frogs (r. esculenta) the lateral parts are innervated from a more caudad situated segment and the medial parts from a more-cephalad segment. In one of the muscles of the upper leg I found exclusively monosegmental innervation, namely, in the semitendinosus. This muscle is only innervated by the 8th nerve.

After these observations were completed, my attention was again directed toward an effort to differentiate the muscle segments with vital dyes. A nerve, for instance the 8th myotome, was stimulated after the intravenous injection of a number of alkaline dyes (phenol-red, methyl-red, bromphenol-blue, congo-red, azolitmine and tintura coccinellae), but in no instance did the lactic acid resulting from muscular activity develop a recognizable change of color in the transversely cut gastrocnemius. The experiment was then repeated using acid dyes (acid fuchsin and "light green"— following the procedure of F. Verzar (1918). Ten or

fifteen minutes after intravenous injection of these dyes the muscle showed a distinct coloration. After this staining of the tissues had developed the 8th spinal nerve was stimulated to complete muscular fatigue several times. Here again inspection for the transverse section of the gastrocnemius failed to show a distinct demarcation between the two muscle segments although the picture was suggestive in a few experiments.

Use was then made of Verzar's observation that muscle stained intravitam with acid fuchsin loses its color when rigor mortis sets in. The procedure was the same as before except that after repeated fatigue of the muscle by stimulation of the 8th spinal nerve, the frog was placed in the thermostat at 37°C. Rigor, of course, developed first in the fatigued muscle. When the belly of the gastrocnemius was cut across after about three hours the area of the 8th myotome showed absolutely white against the pink-colored remainder. The region of the muscle thus delimited is shown in figure 4, in which the white represents the 8th myotome surrounded by the ninth. By the same method the 8th myotome of the tibialis anticus longus, lying on the ventral side of the muscle, can be recognized.

Fig. 4

A second dyestuff, "light green," was also used in this series of experiments. As was the case with acid fuchsin and with the same procedure the eighth myotomal parts of gastrocnemius and tibialis anticus always became whiter after rigor mortis following fatigue from stimulation of the 8th nerve.

In observing these characteristic color changes the semitendinosus, a monosegmentally innervated muscle, was used as a control. When, for example, acid fuchsin was injected and one semitendinosus was completely exhausted by nerve stimulation, it showed distinctly pinker than its unfatigued fellow of the opposite side. If now, however, the frog was placed in the thermostat for three hours the exhausted muscle passed into rigor and was perfectly white while its unfatigued companion was still pinkish in color. Comparable results followed the use of "light green."

Theoretical discussion. It thus appears well established that there is a definite segmental arrangement of the innervation of the muscles of the hind leg of the frog, the ventral (medial) side of the several muscles being innervated by a higher spinal segment than the dorsal (lateral) side. We know from the work of Bolk and Sherrington that the extremities do not

bear a fixed relationship to the central nervous system. This fact was established by observing the innervation of cutaneous areas; sometimes the innervation of a given area shifting by a whole spinal segment. The present investigations show, for the first time, the same situation as regards the muscles. In the case of the muscles the origin of the innervation is not scattered but follows, in every case examined, a perfectly uniform arrangement, i.e., the ventral or median part of any muscle is always innervated from a higher nerve segment than is the lateral or dorsal part. The 8th and 9th spinal nerves always supply the gastrocnemius, for example. But sometimes additional nerve fibres also reach the muscle, sometimes from the 7th and sometimes from the 10th spinal nerves. Whichever of the latter conditions prevails, however, the distribution in the muscle is always uniform; the lower segment always connects the lateral (dorsal) and the higher segment the ventral (medial) portion of the muscle with the central nervous system, while the middle segment controls the mid-region of the muscle. I have likewise demonstrated that this condition is not always strictly bilateral; that is to say, the 7th nerve may be added to the 8th and 9th on one side of the body and not on the other.

It remains to discuss the overlap of these myotomes. We know that the dermatomes overlap to a very considerable extent. In the trunk dermatomes of the cat the present writer (1916) showed that two-thirds of a dermatome may overlap with its neighbour. The method used was to paint the cord about the origin of a posterior root with 1 per cent strychnine sulphate. The area of skin innervated by this particular root could then be readily and accurately outlined because of the heightened irritability. If now an adjacent posterior root was similarly treated with strychnine and the first root sectioned the area of heightened irritability resulting gave an exact pattern of the overlap. In this way one after another dermatome could be mapped out. In general the overlapping was greater on the ventral than on the dorsal surfaces of the body; three posterior roots might overlap on the dorsal surface while four overlapped in certain regions of the ventral surface. This condition presumably has a functional significance; the ventral surface, in the main, receives more stimuli than the dorsal surface, a nice interpretation of which is essential to life.

The question now arises whether the same situation prevails as regards the myotomes. Is there a greater overlap in the innervation of the muscles which have an intensive and complicated function? We may imagine an overlap of myotomes in two ways:

1. We have shown that on the borders the muscle fibres are innervated only from one and the same anterior root. Conceivably the fibres in the mid-region may be innervated by two anterior roots. This is, however, not the case with the gastrocnemius of the frog in which most, if not all, the fibres are monosegmentally innervated (Samojloff, de Boer, Katz). Accord-

ing to Quednau (1926) also the fibres of several muscles with a more complicated function, such as the ext. metacarpi ulnaris of the pigeon and the flexor digitorum communis profundus of the rabbit, are monosegmentally innervated. Hence complication of function does not predicate plurisegmental innervation of muscle fibres. There further investigation is necessary.

2. We may also imagine that the overlap consists of a zone in the middle of the muscle where the fibres, innervated by the various anterior roots are mixed in confusion. Such an arrangement is not inconsistent with the monosegmental innervation of the fibres. Nevertheless I believe that if such an overlap exists in the gastrocnemius it is not very great because in some of my experiments (Pflüger's Archiv, 1926, ccxi, 638, fig. 3) I practically isolated the myotomes. However, I had the impression that in the muscles of the thigh such an overlap was much greater, yet the evidence is insufficient for a definite opinion at the present time.

SUMMARY

1. The lateral (dorsal) parts of the gracilis major, adductor magnus, rectus femoris, abductor magnus, vastus externus and semimembranosus are innervated by the 9th spinal nerve, the medial (ventral) parts by the eighth.

2. If the incision is made not far from the lateral border of these muscles, the lateral strip is exclusively innervated by the 9th spinal nerve. If, however, the incision is made not far from the medial rim, the medial strip is exclusively innervated by the 8th spinal nerve.

3. The semitendinosus is only innervated by the 8th spinal nerve.

4. The muscles can be stained after intravenous injection of acid fuchsin. Sometimes, after stimulation and fatigue of the 8th spinal nerve the piece of the 8th myotome of the gastrocnemius or tibialis anticus longus may be found to be probably redder than the rest. If, after fatigue of the muscle, the frog is hung in the thermostat at 37°C., the 8th myotome has, after about three hours, passed into rigor mortis. This is evident from the white discolouring. The whole of the myotome may be coloured in this way. For vital staining "light green" may also be used.

5. The presence or absence of an overlap of the myotomes is submitted to discussion.

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THE EFFECT OF LACTOSE ON CALCIUM-PHOSPHORUS BALANCE IN DOGS

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Dragstedt (1) and Dragstedt and Peacock (2) were the first to demonstrate that the tetany of parathyroidectomy in dogs may be successfully prevented by use of lactose in the diet. Inouye (3) using Cowgill's diets (4) was able to classify certain diets into groups, those producing tetany and those that prevented tetany. The only variable factor in the diets employed by Inouye was the carbohydrate.

The only well-substantiated metabolic changes that occur in parathyroidectomy are the low blood calcium and the retention of phosphorus. Recently Greenwald (5), (6) has shown that there is a retention of calcium. Collip (7) in 1925 isolated from the parathyroids an active extract which when administered to parathyroidectomized dogs, not only keeps the animal from tetany, but also maintains the level of blood calcium normal or above normal depending on the amount administered. From all recent evidence it seems unquestionable that the tetany caused by removal of the parathyroids is intimately associated with the metabolism of calcium.

In view of the fact that lactose both protects the animals from tetany and maintains the normal blood calcium level (3) it was considered to be of interest to study the calcium and phosphorus balance in both normal and parathyroidectomized dogs fed on lactose-containing diets.

Experimental. Dogs were maintained in metabolism cages. The urine was collected by daily catheterizations and the feces were marked off with carmine. The animals were fed on the experimental diet five to six days prior to the experimental period in order to accustom them to the diet and obviate any effect of previous diet.

Standard methods of analysis were used, calcium was determined by McCrudden's method, total phosphorus by wet combustion and precipitation as the phosphomolybdate, nitrogen by the Kjeldahl method and ammonia by aeration into standard acid. The inorganic phosphates of the blood were determined on whole blood by Briggs' (8) modification of the Bell-Doisy method, and calcium in serum determined according to the method of Tisdall (9). It was found that it was impossible to use a diet

which contained over 15 per cent of lactose due to the violent diarrhea produced by lactose. Even with diets containing only 15 per cent of lactose over 50 per cent of the records had to be discarded due to contamination of the urine with feces. All the animals on the lactose diets were catheterized twice daily[1] as extra precaution against contamination of urine with fecal material.

The diet as fed contained approximately 80 calories per kilo of body weight, which maintained the dogs in a good state of nutrition throughout the experimental period. Enough food was mixed at one time to feed the dog over the entire period. Analyses were made for calcium and phosphorus on each new batch of food. It will be noted in the tables that there is some variation in the calcium and phosphorus content of the various lots of food. This is due to the fact that the casein and bone ash were not always taken from the same lot.

The standard diet used for all the work had the following composition:

Diet 1

	per cent
Sucrose	68.1
Casein	5.5
Bone ash	2.3
Salt mixture	1.2
Butter fat	7.0
Lard	15.9

3 Fleischmann yeast cakes added to each kilo of food.

Diet 2

Same as diet 1 except that 15 per cent of the sucrose was replaced by lactose.

The above diets were found by Inouye (3) to be non-effective and effective respectively against parathyroid tetany.

Table 1 is a representative table of results as obtained in normal dogs. During the lactose feeding period there is an increased calcium excretion and a decrease of total nitrogen and ammonia excretion in the urine. The decrease in total nitrogen is undoubtedly due to the cathartic action of the lactose, preventing absorption of the nitrogenous constituents of the diet. The decreased urinary ammonia was rather unexpected, as the fermentation of lactose in the intestines was believed to cause a more acid reaction of the blood. There is quite a marked loss of calcium and phosphorus from the body during the lactose period in this animal, but no significance can be attached to this, as other animals did not exhibit the

[1] 10 parts sodium chloride
4 parts calcium lactate
4 parts magnesium citrate
1 part iron citrate
A few drops of Lugol's solution

TABLE 1

TABLE 1

Effect of sucrose and lactose feeding on the serum calcium and blood phosphates and calcium and phosphosus balance in the normal dog

Dog—Female, weight 10.4 kilos

DAY	URINE				FECES		INTAKE		TOTAL OUTPUT		BALANCE		BLOOD		DIET
	N	NH_3	P_2O_5	CaO	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	Serum Ca per 100 cc.	PO_4 per 100 cc. whole blood	
	grams	*gram*	*grams*	*gram*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*	
1	2.30	0.1260	0.756	0.0561									10.9	3.22	Diet 1 (sucrose) 175 grams per day
2	2.60	—	0.780	0.0780											
3	2.30	0.0312	0.732	0.0636											
4	2.26	0.1088	0.606	0.0654									11.1	2.75	
5	2.52	0.1044	0.660	0.0780	9.9000	7.8490	2.215	2.310	10.2411	11.3830	+0.8239	+0.1700			
6	2.63	0.0972	0.678	0.0558											Diet 1 (sucrose) 175 grams per day
7	2.23	0.0900	0.712	0.0405											
8	2.52	0.0900	0.782	0.0520									10.9	2.10	
9	2.16	0.0252	0.793	0.0630											
10	1.98	0.0864	0.866	0.0636	10.1599	7.6450	2.215	2.310	10.4348	11.4760	+0.6402	+0.0740			
11	2.16	0.0252	0.658	0.0732									10.7	2.15	Diet 2 (lactose) 175 grams per day
12	2.52	0.1440	1.236	0.1275											
13	1.88	0.0368	0.852	0.1053											
14	2.08	0.0240	0.807	0.1050											
15	1.88	0.0540	0.820	0.1359	11.8760	8.5772	2.215	2.310	12.4229	12.9502	−1.3479	−1.4002	10.7	2.56	
16	2.05	0.0504	0.814	0.1440											Diet 2 (lactose) 175 grams per day
17	1.47	0.0240	0.824	0.0741											
18	2.05	0.0612	0.700	0.1356											
19	2.26	0.0680	0.778	0.1461											
20	1.90	0.0684	0.741	0.1113	11.215	8.4164	2.215	2.310	11.8271	12.2734	−0.7521	−0.7234	10.9	2.09	

TABLE 2

Effect of lactose feeding on the calcium and inorganic phosphates of blood and the calcium and phosphorous balance in the thyroid-parathyroidectomized dog

Dog—Female, weight 12.6 kilos

PERIOD (5 DAYS)	URINE			FECES		INTAKE		TOTAL OUTPUT		BALANCE		BLOOD		DIET	REMARKS
	N	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	Serum Ca per 100 cc.	PO_4 per 100 cc. whole blood		
	grams	*gram*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*		
1st	12.00	0.190	4.617	10.874	7.838	12.65	12.70	11.064	12.455	+1.586	+0.245	–	–	Diet 1 (sucrose) 200 grams per day	Normal
2nd	10.16	0.291	4.572	13.154	7.741	12.65	12.70	13.435	12.313	−0.885	+0.387	11.3	3.40	Diet 1 (sucrose) 200 grams per day	Normal
3rd	8.25	0.650	3.625	13.000	9.436	12.65	12.70	13.650	13.061	−1.000	−0.361	–	–	Diet 2 (lactose) 200 grams per day	Normal
4th	7.55	0.662	3.607	12.168	8.364	12.65	12.70	12.830	11.971	−0.180	+0.729	11.9	3.57	Diet 2 (lactose) 200 grams per day	Normal
5th	7.65	0.406	2.987	11.720	9.616	12.65	12.70	12.126	12.603	+0.524	+0.097	11.1	3.10	Diet 2 (lactose) 200 grams per day	Thyroid-parathyroidectomized
6th	8.16	0.365	4.162	11.650	8.841	12.65	12.70	12.015	13.003	+0.645	−0.293	9.38	3.85	Diet 2 (lactose) 200 grams per day	Thyroid-parathyroidectomized
7th	8.10	0.524	5.213	11.972	8.122	12.65	12.70	12.496	11.339	+0.160	+1.361	9.00	4.00	Diet 2 (lactose) 200 grams per day	Thyroid-parathyroidectomized
8th	10.44	0.433	3.275	12.140	9.497	12.65	12.70	12.573	12.772	+0.077	−0.072	9.25	3.70	Diet 2 (lactose) 200 grams per day	Thyroid-parathyroidectomized

TABLE 3

Effect of lactose feeding on the calcium and inorganic phosphates of blood and the calcium and phosphorus balance in the thyroid-parathyroidectomized dog

Dog—Female, weight 11.9 kilos

PERIOD (5 DAYS)	URINE			FECES		INTAKE		TOTAL OUTPUT		BALANCE		BLOOD		DIET	REMARKS
	N	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	CaO	P_2O_5	Serum Ca per 100 cc.	PO_4 per 100 cc. whole blood		
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm.*	*mgm.*		
1st	11.02	0.120	3.172	12.332	9.212	12.65	12.70	12.452	12.384	−0.198	−0.316	—	—	Diet 1 (sucrose) 200 grams per day	Normal
2nd	10.86	0.236	4.244	11.993	8.136	12.65	12.70	12.229	12.380	−0.421	−0.320	11.8	3.02	Diet 1 (sucrose) 200 grams per day	Normal
3rd	8.59	0.671	3.396	12.912	9.566	12.65	12.70	13.583	12.962	+0.933	+0.262	11.2	3.42	Diet 2 (lactose) 200 grams per day	Thyroid-parathyroidectomized
4th	9.03	0.587	3.874	11.900	8.619	12.65	12.70	12.487	12.493	−0.163	−0.207	10.0	3.81	Diet 2 (lactose) 200 grams per day	Thyroid-parathyroidectomized
5th	8.82	0.603	3.773	10.572	8.744	12.65	12.70	11.175	12.517	−1.475	−0.183	8.8	4.80	Diet 2 (lactose) 200 grams per day	Thyroid-parathyroidectomized

same changes. No changes in blood calcium or phosphates were found, although Inouye (3) found that lactose diets tended to increase the level of blood calcium. Perhaps such increases in blood calcium level would have been found had the experiments been extended over a longer period of time.

In table 2 there is practically no change from the normal in the calcium and phosphorus excretion after parathyroidectomy. There was a significant drop in serum calcium in this dog, but not sufficient to throw the animal into acute tetany. On several mornings before feeding this animal exhibited definite fine tremors, which disappeared in one to two hours after feeding. This animal maintained a fairly good appetite throughout the experiment. The stools were very loose, continually verging on diarrhea. At the conclusion of the experiment the dog was placed on a heavy meat diet and on the third day developed severe tetany and died.

In this experiment there was no fore period of lactose feeding, but the diet was changed from sucrose to lactose on day of operation. This animal refused to eat and had to be forcibly fed throughout the remaining period. Here again the animal was continually on the borderline of tetany. In fact during the 6th period (data not presented) this animal developed a severe attack of tetany which necessitated the administration of 20 grams of calcium lactate by mouth. The animal recovered, but vomited all food on following day so that the experiment was terminated. As in table 2 we see no marked changes in the calcium phosphorus metabolism except the decreased serum calcium.

Discussion. In the results obtained with diets as used by Inouye (3), no marked changes in the calcium and phosphorus balance could be demonstrated. Greenwald (6) has recently shown that there is retention of both phosphorus and calcium in parathyroidectomized dogs, without any necessary change in blood phosphates and a positive decrease in blood calcium.

The animals in tables 2 and 3, in which the thyroid-parathyroid apparatus was removed did not show any retention of either of these elements. In fact the metabolism of these minerals was very similar to that shown by the normal individual on the same diets. Why lactose prevents the retention of these two minerals is hard to explain. It is inconceivable that lactose can supply the hypothetical substance (X) of Greenwald's (6) explanation of parathyroid action. Greenwald (6) has suggested that probably the protective action of lactose is due to the fact that such diets contain a low amount of phosphorus and that the fermentation of lactose produces a more acid media in the gut, resulting in a better absorption of calcium. The fact that the only variable factor in the diets as employed by Inouye (3) was the carbohydrate would speak against the phosphorus content of these diets as the essential feature. Undoubtedly one important factor in the use of lactose is the better absorption of calcium from the gut.

Bergeim (10) has recently shown that diets containing lactose produce better absorption of calcium and phosphorus than other carbohydrate containing diets. The fact that there is an increased calcium excretion, in spite of the more alkaline urine, as shown by the lowered urinary ammonia excretion, indicates a better absorption of calcium on the lactose diets.

The amounts of phosphorus excreted by the kidneys were practically unchanged whether the animal was on the sucrose or lactose diet. Swingle and Wenner (11) have advanced the theory that the reason strontium and magnesium salts prevent tetany in the parathyroidectomized dog is because the gut is rendered less permeable so the loss of calcium from the body is lessened. They further suggest that lactose probably acts similarly. In the experiments reported in the present paper there is no evidence of any such changed permeability. In fact, most of the animals on a lactose diet excreted more calcium, than those individuals on the sucrose diet.

It is interesting that lactose does prevent the retention of calcium and phosphorus in parathyroidectomized dogs, although the mode of action is obscure. The most plausible explanation that can be offered at present is that lactose produces better absorption of calcium from the gut.

SUMMARY

A lactose diet fed to normal dogs produces a diminished ammonia excretion and an increased urinary calcium excretion. In parathyroidectomized dogs lactose prevents the retention of phosphorus and calcium.

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THE HEAT LIBERATED BY THE BEATING HEART

Sixth Communication

The Heat Produced by the Terrapin's Auricle with a Note on the Metabolism During Tonus[1]

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In former papers from this laboratory Snyder (1) has demonstrated the changes in temperature in the surviving ventricle. Since it is well known that the auricle is structurally (2), (3) and functionally different from the ventricle, it was thought advisable to study the temperature changes in the isolated beating auricle and compare them with those of the ventricle.

Method and description of apparatus. The method used was similar to that adopted in the study of the ventricle (1). The heart was removed from the terrapin after ligatures were placed around the tips of the auricles and around the auriculo-ventricular groove, and the ventricle apex cut away. The threads from the tips of the auricles (fig. 1, *e*) were fastened to the thread in the muscle chamber that was later attached to an isometric lever while the ligature around the auriculo-ventricular groove was fastened to the frame of the muscle holder, *f*. The two auricles thus formed a bilobed organ and could be made to enclose a flat-coil thermopile as closely as a pair of sartorius muscles. A thermopile of thirty pairs of iron-constantan junctions, *c*, was inserted between the two auricles and held firmly in place by the close apposition of the tissue covering the "warm" junctions. Pieces of muscular tissue were placed over the "cold" junctions to neutralize the resting effects of the tissue enclosing the "warm" junctions. The preparation was placed in a "vacuum food jar," *a*, after removing all the cracked ice that had been placed in this jar to cool it. The vacuum food jar was then placed in a larger vacuum jar and ice packed in the upper intervening space for temperatures between 5° and 10°C., the amount of space filled with ice varying with the temperature desired. Finally the outer vacuum jar was surrounded with wool-felt and thus held in a third, and larger, container. (See fig. 1 and legend thereto.) In

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this way a fairly constant low temperature was maintained inside the muscle chamber.

This method has been criticised by Fischer (4) who placed two junctions

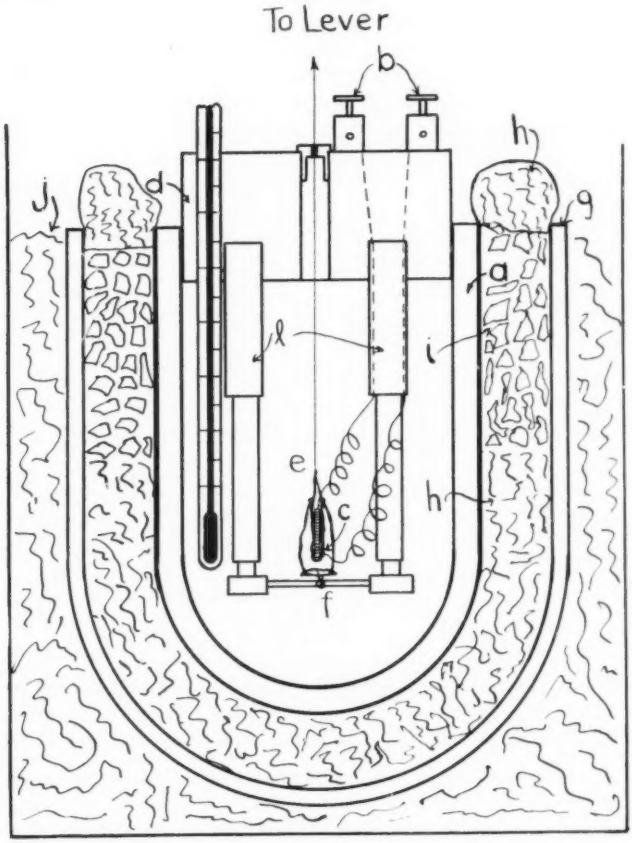

Fig. 1. Muscle chamber. *a*, "Thermos jar;" *b*, binding posts for leading to thermopole and galvanometer terminals; *c*, thermopile of thirty pairs of iron-constantan junctions; *d*, soft-rubber stopper into which supporting frame for the auricles is fastened; *e*, threads from tips of auricle to muscle lever; *f*, rod for attachment of auricle; *g*, vacuum jar; *h*, cotton wool; *i*, cracked ice; *j*, wool felt; *k*, hard rubber posts.

of a single thermocouple in plasticine, the junctions being placed 10 mm. apart. This preparation was suspended in a Dewar flask and the tem-

perature differences noted. He found that at the start of the experiment there was a difference of three degrees, and after four hours a difference of 1.7°C., between the two points. He also states that there was a temperature difference of 20 degrees between the rubber stopper and the bottom of the flask. This experiment of Fischer was repeated using two thermometers calibrated by the Bureau of Standards. One thermometer was placed in the center of the flask in the same position that the muscle occupies during the experiment and the second thermometer was placed to one side, the bottom of the bulb of the middle thermometer being 10

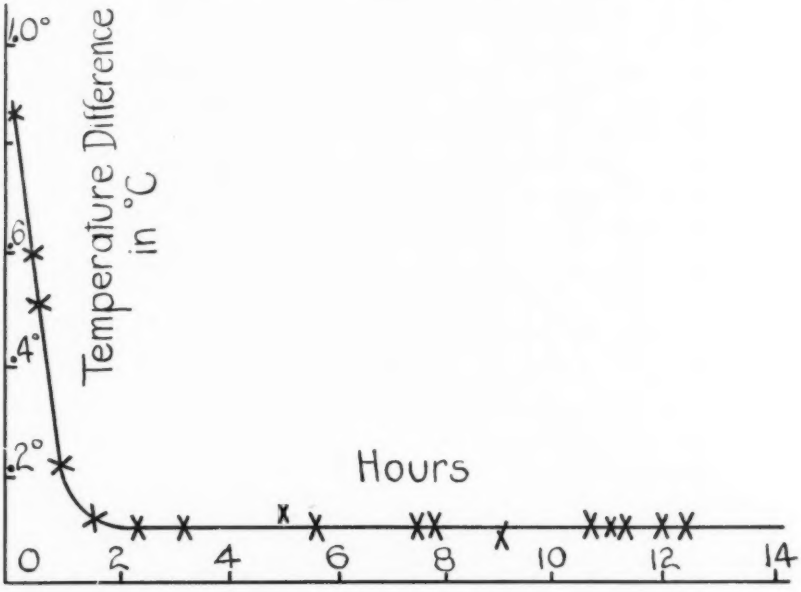

Fig. 2. Temperature differences in 0.1°C., at two points 10 mm. apart in the muscle chamber, plotted against time in hours.

mm. above that of the side thermometer and the temperatures of the two thermometers were noted over a period of thirteen hours. The results were plotted in figure 2. From the curve it is apparent that the temperature differences between the two thermometers quickly reaches a minimum of 0.1 degree and this difference is maintained over a long period of time. Between two points 50 mm. apart a temperature difference of 1.6°C. was noted. Knowing that a temperature difference does exist in the different levels of the flask, an experiment was tried to see if this temperature difference was sufficient to give thermal disturbances due to the

change of the position of the auricle during contraction. At the end of the experiment of November 30 the preparation was killed by chloroform and the method of Snyder (5) was used to estimate the effect of the differences in temperature in the flask. Weights were hung on the lever to lower the thermopile, the amount of lowering of the thermopile corresponding to the lowering of the preparation during the systole of the auricles. With a deflection of 2 mm. on the smoked drum, an actual lowering of the thermopile of 0.197 mm., the galvanometer gave a deflection of 6 mm. in 48 seconds, or a temperature difference of 1.5×10^{-5} calorie per mm.

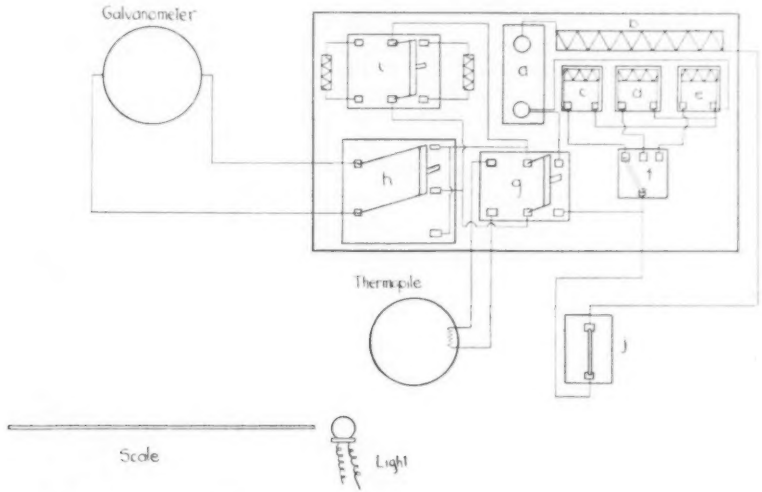

Fig. 3. Diagram of connections. *a*, Standard cell; *b*, 100,000 ohm resistance; *c*, 0.1 ohm shunt; *d*, 0.2 ohm shunt; *e*, 0.3 ohm shunt; *f*, three-way switch for connecting shunts across circuit; *g*, double throw switch for connecting galvanometer to thermopile circuit or to battery circuit; *h*, pole changer; *i*, double throw switch for placing shunts for 1 or 2 ohms in parallel with galvanometer circuit.

deflection per second. Using the factor 7/12 (Snyder, 5) the correction value is 0.875×10^{-5} calories per millimeter deflection per second. The average duration of the cardiac systole was nine seconds with a shortening of 0.29 mm., giving a correction of $9 \times 0.29 \times 0.875$ or 2.28×10^{-5} calorie. In this experiment the average heat produced was 292×10^{-5} calorie, the corrected value 289.72×10^{-5} calorie, a negligible correction of 0.78 per cent for contractions of short duration. But since this correction is a function of the time as well as the displacement, the correction when applied to the tonus changes in the experiment of March 12, 1926, are no longer negligible. In this experiment the tonus waves were of such

TABLE 1

NUMBER OF THERMOMTOGRAM	MAXIMUM HEAT PRODUCED PER GRAM OF AURICLE CALORIES $\times 10^{-5}$ (H_t)	INITIAL TENSION PER GRAM OF AURICLE (i)	TOTAL TENSION PER GRAM OF AURICLE (T)	H_t/T
I 1A	340.0	18.33	28.2	12.1
1B	337.0	18.33	28.2	11.9
1C	340.0	18.33	28.2	12.1
1D	325.0	18.33	28.2	11.5
2A	246.0	18.33	28.2	8.73
2B	270.5	18.33	28.2	9.62
2C	312.0	18.33	28.2	11.05
2D	348.0	18.33	28.2	12.32
4A	256.0	21.55	29.8	8.6
4B	253.0	21.55	29.8	8.5
4C	342.0	21.55	29.8	11.5
II 3a	45.4	1.17	4.2	10.8
3b	49.8	1.17	4.2	11.8
3c	47.3	1.17	4.2	11.5
III 1a	91.2	11.4	13.44	6.8
1b	82.5	11.4	13.44	6.14
1c	85.5	11.4	13.44	6.36
1d	89.4	11.4	13.44	6.64
1e	95.2	11.4	13.44	7.08
IV 1a	234.0	26.2	31.8	7.35
1b	268.0	26.2	31.8	8.42
1c	261.0	26.2	31.8	8.20
1d	246.0	26.2	31.8	7.73
2a	232.0	25.4	30.0	7.75
2b	232.0	25.4	30.0	7.75
2c	225.0	25.4	30.0	7.5
2d	242.0	25.4	30.0	8.05
2e	232.0	25.4	30.0	7.75
2f	266.0	25.4	30.0	8.87
2g	240.0	25.4	30.0	8.0
3a	312.0	30.3	33.1	9.43
3b	314.0	30.3	33.1	9.5
3c	313.0	30.3	33.1	9.44
3d	312.0	30.3	33.1	9.4
3e	310.0	30.3	33.1	9.36
3f	314.0	30.3	33.1	9.5
3g	314.0	30.3	33.1	9.5
4a	368.0	35.0	38.8	9.48
4b	363.0	35.0	38.8	9.36
4c	370.0	35.0	38.8	9.55
4d	363.0	35.0	38.8	9.36
4e	366.0	35.0	38.8	9.42

I. Experiment of November 17, 1925. Weight of auricles, 0.372 gram. Temperature of muscle chamber for 1, *ca.* 7.0°C.; 2, *ca.* 5.4°C.; 4, *ca.* 8.0°C. Deflection of galvanometer read directly on the scale and the early phases of the thermal response, the mechanical response and the time intervals were photographed. Auricles beating once every 30 seconds. Records taken from two to four hours after the muscle was placed in the chamber.

II. Experiment of April 7, 1926. Weight of auricles, 1.05 gram. Temperature of muscle chamber *ca.* 6°C. Thread galvanometer used with aluminum thread, 5 micra in diameter of 226.3 ohms resistance. Photographs taken of thread deflection, mechanical response and time intervals. Auricles beating once every 13 seconds (see fig. 4). Records taken *ca.* 3 hours after muscle was placed in the chamber.

III. Experiment of October 26, 1926. Weight of auricles, 0.57 gram. Temperature of muscle chamber *ca.* 5.6°C. Deflections of galvanometer, mechanical response and time intervals recorded on smoked drum. Auricles beating once every 30 seconds. Records taken two hours after muscle was placed in chamber.

IV. Experiment of November 30, 1926. Weight of auricles, 0.66 gram. Temperature of muscle chamber 5.0°C. Deflections of galvanometer, mechanical response and time intervals recorded on smoked drum. Auricles beating once every 42 seconds. Records taken from one to two hours after muscle was placed in chamber.

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duration that the correction was from 40 per cent to 50 per cent of the observed value.

In each experiment a thermometer was inserted through the cover so that the approximate temperature of the muscle chamber could be observed. Through a double-throw switch (fig. 3, *g*) the galvanometer was connected to the thermopile circuit or to the calibrating set. The calibrating set for the galvanometer consisted of a standard cell, *a*, connected in series to a 100,000 ohm resistance, *b*, and was shunted through a three-way switch to 0.1, 0.2, 0.3 ohm shunts, *c*, *d*, *e*, the calibrating circuit being closed by switch *j*. Across the galvanometer circuit was placed the double throw switch, *i*, so that shunts of one or two ohms could be placed in parallel with the circuit. A pole changer, *h*, was placed in the circuit so the direction of the calibration current was maintained in the same direction as the positive heat production. The galvanometer was of the d'Arsonval type with a sensitivity of about 45 mm. to 5.5×10^{-8} ampere, and a whole period of *ca* 24 seconds, with a critical damping resistance of 12 ohms. The record of the galvanometer deflection was taken on a revolving sensitive film at a distance of one meter. On the same film were taken photographs of the mechanical response of the auricle and also the time in intervals of three seconds' duration. In some of the experiments the galvanometer deflections were of small enough magnitude so that they also could be photographed, but when the deflections exceeded the shutter opening of 60 mm. the total deflection was read directly on a scale. The auricles were attached to a short isometric lever of the fret-saw type, a mirror being fastened to the tip of the lever so that the changes in tension could be photographed. A metronome recorded the time intervals by an

electrically controlled lever and periodically interrupted a beam of light. The galvanometer was calibrated at the beginning and end of each record, and the tension-lever before each experiment. The results were converted into absolute units, calculating by the indirect method (6).

In experiment II, the Einthoven thread galvanometer was used in place of the d'Arsonval galvanometer. Herlitzka (7) used a thread galvanometer for the determination of the thermal changes in the isolated rabbit's

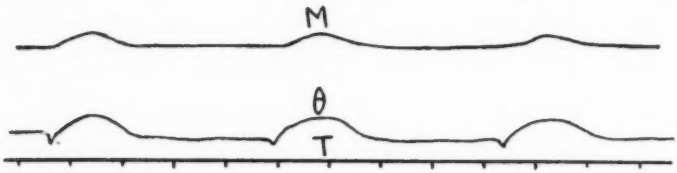

Fig. 4. Experiment of April 7, 1926. Temperature of the muscle chamber *ca* 6°C. Thread galvanometer used with an aluminum thread of 226.3 ohms resistance. Line tracing of photograph scale 2:5 shows mechanical response, M, thermal response, θ, time intervals, T, of three seconds each and deflection of thread to calibration current. Small negative deflection of thread coming before systole probably due to action current, escaping through a break in the paraffin insulation. Calibrating current from standard cell of 1.018 volts with 100,000 ohms in series and with 0.1 ohm shunt gave 28 mm. deflection. Muscle placed in chamber at 11:40 a.m. Record taken at 3:15 p.m.; 1.3×10^{-8} ampere through galvanometer gave 27 mm. deflection, or 1 mm. is equal to 5×10^{-10} ampere. Since there are 30 pairs of junctions, each junction giving 53×10^{-6} volt for 1° difference, there would be $\frac{30 \times 53 \times 10^{-6}}{13.64 + 226.3} = 6.6 \times 10^{-6}$ ampere for one degree difference in temperature between the "warm" and the "cold" junctions. (The denominator is the sum of the resistances of the thermopile-galvanometer circuit.)

$\frac{5 \times 10^{-10}}{6.6 \times 10^{-6}} = 7.6 \times 10^{-5}$ degree C. or 6.3×10^{-5} calorie for 1 mm. deflection or 50.4×10^{-5} calorie for 8 mm. deflection.

Total tension equals the initial tension plus the rise in tension or $1.17 + 3.03 = 4.2$ grams of tension per gram of muscle

$\frac{50.4 \times 10^{-5}}{4.2} = 12.0 \times 10^{-5}$, the value for H_t/T.

heart. Since the resistance of the threads used in his experiments was high, the response of the thread to the thermal changes was very small. Using the method developed for obtaining thermomyograms with thread galvanometers (Snyder and Gemmill, 8), greater sensitivity was obtained in this experiment.

Results. The results of the experiments are given in table 1. In this table the maximum heat produced per gram of muscle, H_t, is compared with the total tension developed in grams per gram of muscle, T. The initial

tension is also given in grams per gram of muscle. In the last column the ratio of the heat produced to the tension developed, H_t/T is given.

The experiment of November 17 gave eleven records. The galvanometer deflections were read directly from a scale,—the beginning of the thermal response being photographed together with the mechanical response and the time intervals. In the last part the initial tension was increased thereby increasing the total tension. In the first part there is very little deviation of the ratios of H_t/T, the average being 11.9 and giving a deviation of +0.2 and −0.4, or a maximum deviation of 3 per cent. In the second part taken one hour and half after the first part a greater deviation is observed, the average ratio being 10.43 with a deviation of +1.89 and −1.7, or 18 per cent. In the last part taken three hours

TABLE 2

Average values

		MAXIMUM HEAT PRODUCED PER GRAM OF AURICLE CALORIES × 10^{-6} (H_t)	TENSION PER GRAM OF AURICLE (T)	H_t, T
I	1	335.5	28.2	11.9
	2	294.1	28.2	10.43
	4	283.6	29.8	9.5
II	3	47.5	4.2	11.3
III	1	88.76	13.44	6.6
IV	1	252.0	31.8	8.0
	2	238.0	30.0	7.9
	3	313.0	33.1	9.4
	4	366.0	38.8	9.4

and twenty minutes after the first record the ratio of H_t/T gives an average of 9.5 with a deviation of +2.0 and −1.0, or 21 per cent. (See I, table 1.)

In the experiment of April 7, 1926, the Einthoven thread galvanometer was used with a low resistance aluminum thread. The record obtained is shown in figure 4. A small negative deflection is seen before each positive wave, the thread returning to a base line before the positive wave is recorded. This initial negative deflection is probably due to the action current of the muscle, which must have leaked through a break in the paraffin insulation of the thermopile. The analysis of the record gave results comparable to the results obtained with the d'Arsonval galvanometer, the ratio of H_t/T varying from 10.8 to 11.8, the average being 11.36, giving a deviation of +0.44 and −0.56, or 3.9 per cent. (See II, table 1.)

For the experiment of October 26, 1926, the d'Arsonval galvanometer

was used and its deflections recorded on a smoked drum. No negative deflections were seen in this experiment. Five readings were taken, the ratio of H/T for the determinations varied from 6.14 to 7.08, giving an average ratio of 6.6 for the experiment. (See III, table 1.)

In the experiment of November 30, 1926 (IV, table 1), the initial tension was changed from 25.4 grams per gram of muscle to 35 grams per gram of muscle in four stages. The resulting heat production was compared with the tension developed and the ratios of the heat produced per gram tension per gram muscle varied from 7.9 to 9.4. The average results for the preceding experiments are given in table 2.

There is still one other experiment whose results have been reserved to the last for special description. This is done because they include the

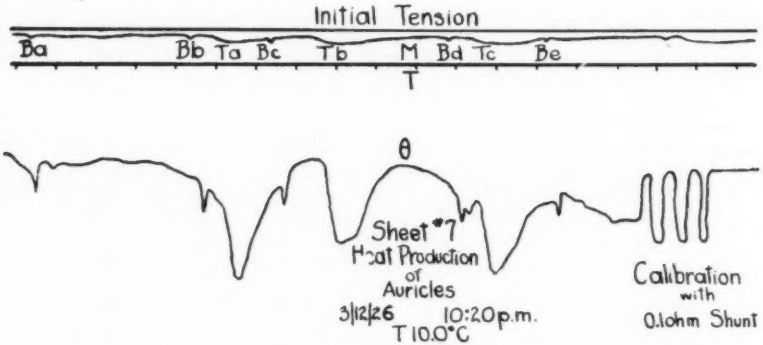

Fig. 5. Experiment of March 12, 1926. Line tracing of smoked drum record, scale 1:3. d'Arsonval galvanometer used with internal resistance of 18 ohms. Record shows initial tension, auricular beats, B, and tonus changes, T, with corresponding heat changes, θ, in muscle.

observation of a remarkable phenomenon that had not been noticed in any of the other preparations, and so far as the writer can find out, a phenomenon that has not been observed by anyone else.

In this experiment of March 12, 1926, as in some of the others, the d'Arsonval galvanometer was used and the responses recorded on a smoked drum.

The preparation had been placed in the heart chamber at 2:30 p.m. and the usual type of deflections had been obtained that have been described in the preceding paragraphs. But at 7:15 p.m. it was noticed that the auricles were undergoing a peculiar rhythmic tonic change that was totally separated from the regular beats, and that with the tonus waves there were corresponding thermal responses. Several records were obtained, a typical record is shown in figure 5. In this record the auricle

beats normally, *Ba*, and there is an immediate thermal response, then there is a long pause followed by another normal auricular beat, *Bb*, with a corresponding thermal response; then the auricles undergo a tonic change,

TABLE 3

NUMBER OF THERMOMYOGRAM	MAXIMUM HEAT PRODUCED PER GRAM OF AURICLE CALORIES 10^{-5}	CORRECTION	CORRECTED VALUE	TOTAL TENSION PER GRAM OF AURICLE	H_t/T
Experiment V					
No. 4 Ta* ...	100.0	47.0	53.0	9.32	5.7
Ba.....	40.3			9.44	4.27
Tb.....	80.5	44.7	35.8	9.96	3.6
No. 5 Ta....	136.3	55.7	80.6	9.05	8.92
Ba....	19.8			9.04	2.19
Bb....	39.7			7.33	5.41
Tb....	99.3	42.8	56.5	9.32	6.07
Bc.....	32.2			6.63	4.86
Tc.....	104.0	48.2	55.8	7.97	6.9
No. 6 Ta....	119.0	50.0	69.0	9.33	7.4
Tb....	122.0	53.5	68.5	9.96	6.87
Ba....	18.9			9.93	1.92
Tc.....	93.3	38.3	55.0	10.11	5.4
Bd....	17.38			8.1	2.14
Td....	121.6	44.8	76.8	10.11	7.6
Bc.....	17.38			7.97	2.18
No. 7 Ba....	19.83			8.27	2.4
Bb....	27.2			7.32	3.71
Ta....	117.6	53.0	64.6	8.76	7.31
Bc.....	23.6			9.28	2.54
Tb....	168.0	43.0	125.0	9.96	12.7
Bd....	20.6			8.00	2.58
Tc.....	96.8	51.5	45.3	9.32	4.9
Bc.....	17.38			9.05	1.92

V. Experiment of March 12, 1926. Weight of auricles, 0.47 gram. Temperature of muscle chamber, *ca* 7.5°C. Record of mechanical response, thermal response and time intervals taken on smoked drum (fig. 5). Separation of auricular beats from tonic contractions with the corresponding thermal changes.

* In the symbols of this column T refers to auricular tonic contractions and B to auricular beats. Records taken from five to eight hours after muscle was placed in the chamber.

Ta, and immediately there is a marked thermal response. While the auricles are slowly relaxing from the tonic contraction, there is an auricular beat, *Bc*, with a similar thermal response as obtained in the preceding

beats. The next contraction is purely a tonic contraction, *Tb*, and a large thermal response is registered. In the next cycle we have similar conditions to the first cycle, an auricular beat, *Bd*, followed by a tonic wave, *Pc*, and another auricular beat, *Be*, during the relaxation of the tonic wave. The duration of the auricular beat is *ca* 4.5 seconds and for the tonic contraction *ca* 180 seconds.

The values for the heat produced with corrections and the tension developed per gram of muscle are given in table 3, and the corrected values of the heat produced as compared to the tension developed during the

TABLE 4

Experiment V. March 12, 1926

	AURICULAR TONIC CONTRACTIONS H_t/T		AURICULAR BEATS H_t/T	
No. 4	Ta	5.7	Ba	4.27
	Tb	3.6		
No. 5	Ta	8.92	Ba	2.19
	Tb	6.07	Bb	5.41
	Tc	6.9	Bc	4.86
No. 6	Ta	7.4	Ba	1.92
	Tb	6.87	Bb	2.14
	Tc	5.4	Bc	2.18
	Td	7.6		
No. 7	Ta	7.37	Ba	2.40
	Tb	12.7	Bb	3.71
	Tc	4.9	Bc	2.54
			Bd	2.58
			Be	1.92
Average		6.9		3.01

beating of the auricles and during the tonic contraction are given in table 4. This value of the heat produced to the amount of tension exerted during tonus is a constant and averages 6.9 and for the auricular beats the average is 3.01. The value for the auricular beats obtained in this experiment does not agree with the value obtained in the other experiments but the records in this experiment were taken from five to eight hours after the muscle was placed in the chamber while in the former experiments the records were taken from two to four hours so an exact comparison is not permissible. The outburst of heat during the tonic contraction must signify metabolism in the muscle, probably in the smooth muscle fibers of which the terrapin's auricular has an abundant

supply (2), (3). The value obtained for H/T for the tonic waves agrees closely for the value obtained by Snyder (5) for the contraction of the cardiac sphincter of the terrapin's stomach. Thus the records show the heat produced by the auricular beats and the metabolism during tonus but an exact comparison of the thermal responses of the two different types of contraction is impossible as the relative proportion of the cardiac fibers to the smooth muscle fibers is not known. Parnas (9) and Bethe (10) have shown that the oxygen consumption during tonus is not increased and Evans (11) has shown that the oxygen consumption during tonus is actually less than that of the resting muscle and increases during the relaxation of the muscle. Since this experiment demonstrates heat produced during tonus, this heat is probably in part produced by an anerobic chemical reaction in the smooth muscle fibers of the auricle, the nature of the production of heat being similar to the anerobic stage in the metabolism of skeletal muscle; for Evans (12) has shown that lactic acid may be produced in smooth muscle under the same conditions that produce lactic acid in skeletal muscle. The remainder of the heat is probably produced by an oxidative combustion of part of the formed lactic acid (Evans, 13).

When the various average values of H_t/T are considered (table 2), the total average ratio for all the experiments is 9.4, giving a maximum and minimum deviation of $+2.5$ and -2.8 respectively.

The results in a study of the ventricle (Snyder, 1, p. 269) indicate the average value of H_t/T for the ventricle to be 10.5, a number agreeing with the ratio obtained here for the auricle. From this it appears that the auricle and ventricle both produce about the same amount of heat per gram of muscle per gram of tension developed. Similarly Hartree and Hill (14) find a constant ratio for the isometric contractions of the sartorius muscle of the frog.

I wish to thank Prof. C. D. Snyder for his many helpful suggestions and criticisms.

SUMMARY

The heat production of the terrapin's isolated, spontaneously beating auricle was studied, and from the results it appears that:

a. During each beat the ratio of the total heat produced per gram of muscle per gram tension developed approaches a constant.

b. The ratio has an average value of 9.4×10^{-5} calories.

c. The value of this ratio agrees with that obtained for the terrapin's ventricle.

d. During one experiment, there was a separation of the normal auricular beats from the tonic contractions of the auricle; thermal changes were observed corresponding to these pure tonic waves which seemed to demonstrate a heat production during tonus.

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STUDIES ON METABOLISM

V. Inactivity and Age as Factors Influencing the Basal Metabolic Rate of Dogs

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The importance of regular muscular activity for the maintenance of health seems to be demonstrated by practical experience. The changes in physiological processes during exercise have been the subject of much experimental analysis.

The literature bearing upon the relationship between the gaseous exchange, the output of mechanical work, and the mechanical efficiency of the human body has been reviewed by Benedict and Catheart (1913). Other problems relative to the output of the heart, the arterial blood pressure, the passage of oxygen into the tissues, and the coördination of the changes occurring during exercise, have been reviewed by Bainbridge (1919), and more recently Hill (1926) has discussed in detail the chemical changes accompanying muscular activity. The investigations mentioned above deal almost entirely with the phenomenon of muscle contraction, or with the changes that occur during a very short period after the gradual cessation of the muscular work.

Marked muscular inactivity has been less thoroughly investigated. For example, no reference could be found in the literature on the influence of prolonged periods of restricted activity on the basal metabolic rate of normal, non-hibernating animals. In the present report, data are presented showing the basal metabolism of 4 dogs under different conditions of physical activity, and varying in age from young adults to extreme old age.

Plan of the experiment. The four dogs used in this research are designated numerically according to their chronological position in our metabolic series. At the beginning of the experiment, dogs II, V and IX were young adults between one and two years old. They had been housed in the laboratory under dietary management for a period of time sufficient in duration to determine their maintenance requirements. Dog VIII was an old obese female that had been in this laboratory for 12 years prior to these

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observations. During the experiment, dogs II, V and IX received carefully determined high protein maintenance rations. Dog VIII was fed table scraps *ad libitum*. Observations on the basal metabolism of dog II were in progress nearly 4½ years (July 29, 1921 to January 6, 1926); on dog V for approximately 3 years (April 1, 1923 to February 22, 1926); on dog IX for 6 months and on dog VIII for 2 months. The interval of time that each

TABLE 1

Data taken from the protocols of dog II, showing the basal metabolism during periods I (standard amount of activity), II (rigorously restricted activity), and III (reinstatement of daily exercise)

PERIOD DATE	WEIGHT	TOTAL CALORIES PER 24 HOURS	PERIOD DATE	WEIGHT	TOTAL CALORIES PER 24 HOURS
	kgm.			*kgm.*	
Period I			Period II continued:		
1921			*1925*		
August 23	12.4	450	October 19	14.8	432
August 24	12.3	476	October 20	15.0	447
August 25	12.4	470	October 21	14.9	438
			October 22	14.9	447
1923			November 1	14.8	445
February 23	13.1	488			
February 24	13.1	482	Period III		
February 28	13.1	483	*1925*		
			December 17	15.0	535
November 4	13.1	496	December 18	14.9	537
November 5	13.0	480	December 19	14.9	524
November 6	13.2	484	December 20	15.0	529
November 7	13.2	488	December 21	15.0	533
			December 22	14.9	538
Period II			December 23	14.9	532
1925			December 24	15.0	525
August 29	15.0	431	December 25	15.0	536
August 31	14.8	435	December 26	15.0	505
September 1	14.8	430			
September 16	15.0	436			
September 29	14.9	430			

dog was studied is divided into periods corresponding to changes in physical activity, namely—period I, characterized by the standard amount of muscular activity (Kunde and Steinhaus, 1926); period II, rigorously restricted physical activity; period III, gradually, increasing amounts of exercise following a period of restricted activity. The technic employed in this laboratory for determining the basal metabolism of dogs has already been described (Kunde, 1923).

Results. Space will not permit a detailed publication of all the data accumulated since, in most instances, daily basal metabolism determinations were made during the entire time that the dogs were under observation. Consequently, for dogs V and IX only the results of each period based on an average of 10 or more daily metabolism determinations are given. Table 1 contains data taken from the protocols of dog II, at widely separate intervals, and representing each period. The first period for this dog was two years and four months in duration (July 29, 1921 to Nov. 15, 1923). The average results of the 3 tests made during August, 1921, show that the basal metabolism at this time is 775 calories per square meter of body surface per 24 hours. The average for the 3 tests of February,

TABLE 2

Showing the basal metabolism of dog VIII, female. A very old dog with laboratory records extending over 12 years. The exact age of this dog is not known*

DATE	BODY WEIGHT	BODY TEMPERATURE	PULSE PER MINUTE	RESPIRATION PER MINUTE	CALORIES OF HEAT PER SQUARE METER PER 24 HOURS
1926		°F.			
March 5	15.4	100.6	56	10	546
7	15.4	100.6	72	16	530
8	15.3	101.0	70	16	541
9	15.2	100.8	70	16	524
14	15.5	100.6	68	16	543
15	15.4	100.6	68	12	564
16	15.4	100.6	68	14	568
17	15.4	101.4	70	14	568
18	15.4	100.8	62	14	549
22	15.4	100.8	72	12	524

* The surface area computed from the formula $\sqrt[3]{W^2} \times 0.112 = 0.693$ sq. m. The heat production, based on the average of the 10 tests is 778.4 calories per sq. meter of body surface per 24 hours.

1923, is 778 calories, and for the 4 tests of November, 1923, is 783 calories. The second period (rigorous restrictions in muscular activity) was 22 months in duration (January 1, 1924 to November 1, 1925). The average for the 10 tests given for this period shows the metabolism to be 646 calories per square meter per 24 hours; and for the third period (increasing amounts of daily exercise), November 1, 1925, to Jan. 6, 1926, the metabolism averages 782 calories.

The first period for dog V extended from April 1, 1923, to October 4, 1925. The average of 10 daily metabolism determinations at three widely separate intervals show the following variations: from April 1 to 11, 1923, the surface area is 0.503. The basal metabolism in terms of calories of

heat produced per square meter of body surface per 24 hours is 695. From November 11 to 21, 1924, the surface area is 0.526; the basal metabolism 696 calories. This dog was not subjected to a period of vigorously restricted activity. During the period of increasing amounts of daily exercise (October 4, 1925, to February 22, 1926) the surface area is 0.557; the heat production 777 calories.

The period of unrestricted physical activity for dog IX extended from September 1, 1924 to May 11, 1926. Near the end of this time (April 18 to May 11, 1926) the basal metabolism averaged 688 calories per square meter per 24 hours. The surface area is 0.67 square meter. After daily exercising had been allowed for 5 months, the metabolism rose to 846 calories and the surface area increased to 0.705 square meter. Table 2 contains data showing the daily basal metabolism of a very old obese dog after 12 years of laboratory life.

Discussion. The difference in the age and sex of the several dogs, variations in the length of the periods, and individual methods used for encouraging increasing amounts of daily muscular exercise make it impossible to group the collective results of each period. Consequently, a detailed discussion of the results of dog II is given and only significant variations from these findings considered for the other dogs.

No change occurred in the basal metabolism of dog II during the first $2\frac{1}{2}$ years of its residence in the laboratory, but during this time it was housed in a booth with sufficient space for moderate exercising and in addition was allowed to run in a large room with other dogs for 2 hours daily. This together with a high protein high calorie maintenance diet was sufficient to keep the metabolism constant (within variations of 1 per cent), when comparisons are based on 10 daily (15 minute period) determinations. The lowered metabolism occurred after every available means had been employed to discourage muscular activity. It was confined in a small cage, carried to the metabolism room for the daily tests, and never allowed to voluntarily leave the cage. The success of the experiment at this stage was due largely to the phlegmatic disposition of the dog, without which no assurance of a reduction in muscular exercise could have been made, despite the close confinement, as it is well known that many irritable dogs exercise vigorously in their manifestations of resentment to cage life. This dog was a fat animal before the activities were restricted and with a gain in weight of 2 kilos during that time, it appeared a very obese, but otherwise healthy well kept dog, with thick even glossy coat, and skin entirely free from pathological changes.

The essential factors which may have contributed to lowering the heat production seem to be: *1st, age;* nearly 4 years have passed since metabolism observations were first made on this dog. These 4 years in the life time of a dog correspond approximately to the age range of 20 to 40 years

in man. It has been shown that for man there is a decline in the heat production between these years of approximately 15 per cent (Harris and Benedict, 1919). But with this dog the decrease reached 17 per cent in 22 months, followed by a correspondingly great increase in 9 weeks. This reversal of the direction of change in the metabolism is entirely out of keeping with the decline that occurs throughout the whole range of adult life in man. More evidence indicating that age exerts a negligible influence on the basal metabolism of dogs is contained in the data of table 2. This shows the heat production of dog VIII (♀) an obese animal which had reached extreme old age. Dog VIII was a fullgrown adult, not less than 2 years old when it first became a resident of this laboratory, 12 years prior to the observations of this report, yet the heat production is well within the limits of that which seems normal for middle aged adult dogs. Furthermore, within the past year, numerous metabolism determinations have been made on a dog of unknown age, but the loss of teeth and other signs of deterioration give evidence of its extreme old age, yet the basal metabolism is 802 calories per square meter per 24 hours. These results suggest that advancing age cannot be considered a factor in influencing the basal metabolic rate of dogs after growth in stature has ceased.

2nd. Changes in environmental conditions, such as direct sunlight and out-of-door air. The powerful influence of the direct rays of the sun on some metabolic processes in higher mammals, makes it necessary to consider the environmental conditions during the different levels of metabolic activity. The cage in which the dog was confined during the period of low metabolism was in the same remote part of the laboratory as the booth occupied. This was well ventilated, steam heated but entirely free from the direct rays of the sun. The treadmill used for encouraging exercise during the 3rd period was in the cellar. The dog at no time was allowed to run out-of-doors. A seasonal variation if present would exert equal influence on both normal and decreased metabolism since both conditions extended over many months.

3rd. The caloric intake and changes in body weight. At the beginning of the first period and the entire second and third periods, the dietary remained unchanged (250 grams meat, 100 grams bread, and 250 cc. whole milk). But during the second period the body weight increased to 15 kilos and remained so throughout the third period. This is a gain of 19 per cent over the initial weight, consequently, the caloric intake per kilo of body weight is proportionally diminished. If we ignore the lowered caloric intake and the controlled conditions of physical activity, we have, at the end of the second period, a very fat animal with changes in body weight and basal metabolic rate (in terms of calories per square meter of body surface) almost inversely proportional to each other, which suggests a less active mass of protoplasmic substance made up largely of storage tissue,

hence a lowered metabolism due to adiposity. But Rubner (1902) showed that simple obesity in man does not change the basal metabolism. This finding was confirmed by Means (1915), and later, Strouse et al. (1924) concluded that overweight is not associated with a constant change in the

TABLE 3

Comparison of the basal metabolism of young and old dogs under different conditions of physical activity. The surface area is computed from the formula $\sqrt[3]{W^2} \times 0.112$

NUMBER OF DOG SURFACE AREA TIME	APPROXIMATE AGE	CONDITION OF PHYSICAL ACTIVITY	CALORIES OF HEAT PRODUCED PER SQUARE METER OF BODY SURFACE PER 24 HOURS	PER CENT DEVIATION FROM THE BASAL METABOLIC RATE OF NORMAL DOGS*
Dog II, male 0.6 sq. meter 1921, Aug. 23 to 26	Young adult	Standard amount of laboratory activity	775	+0.5
Dog II, 0.675 sq. meter 1925, August 29 to November 1		After 22 months of rigorously restricted activity	647	−16.1
Dog II, 0.675 sq. meter December 17, 1925, to January 6, 1926		After 9 weeks of daily indoor exercising	782	+1.5
Dog V, male 0.505 sq. meter 1923, April 1 to 12	Young adult	Standard amount of laboratory activity	695	−0.9
Dog V, 0.557 sq. meter 1926, January 13 to February 22		Unrestrained freedom	777	+0.7
Dog VIII, female 0.697 sq. meter 1926, March 5 to 23	Extreme old age	After 12 years of laboratory life	783	+0.9
Dog IX, female 0.67 sq. meter 1926, April 18 to May 12	Young adult	After 20½ months of restricted activity	688	−10
Dog IX, 0.705 sq. meter 1926, October 1 to 13		After 5 months of daily out-of-door exercising	846	20.97

* Kunde and Steinhaus (1926).

metabolism. Moreover, during the third period, after moderate exercising was again encouraged, the body weight remained constant, whereas the basal metabolism increased to its initial level. The ability of a fat animal to maintain a constant body weight under what appears to be an unequal distribution between the caloric value of the food ingested, and the addi-

tional energy spent by the work performed during increasing amounts of daily exercise is not contrary to the law of the conservation of energy, because during this process muscle tissue is developed from the nitrogen containing substance of the diet. The material necessary to supply the energy for metabolism is possibly derived from the excess body fat.

The greatest gain in weight of dog II occurred during the period of restricted activity and low basal metabolism. But increasing the muscular activity does not represent a simple reversal of the internal processes, since increasing the daily exercise did not result in a corresponding loss in body weight, though the basal metabolism increased to its initial level. The work of Grafe and Graham (1911) is of special significance in this respect. These authors made a metabolic study of a dog which lasted 107 days. The initial weight of the dog was 21.15 kilos. During a hunger period of 7 days the weight decreased to 15 kilos. The body weight returned to its initial level after 7 days of realimentation. The dietary at this time had a caloric value of 280 per cent of the minimal basal requirements. Following this for a period of 29 days the daily caloric intake was 300 per cent of the minimal basal requirements with a gain in weight of only 0.3 kilo. For the next 11 days the food ingested was 200 per cent of the minimal basal requirements with a loss of 0.13 kilo. Next, for a period of 19 days, the caloric intake was 130 per cent of the basal requirements and the loss in weight only 0.1 kilo. The dietary was reduced once more and the study closed with a hunger period. Moulton (1915), making similar observations in cattle, concluded "that the digestive coefficient increases as the plane of nutrition is lowered."

4th. The influence of muscular activity. The physical condition of dog II at the close of the period of confinement is best described as a state of chronic fatigue. It was encouraged to gradually increase the daily activity by compelling it to run a treadmill, at moderate speed, for 15 minutes to 1½ hours daily. Exertion to the point of exhaustion was always guarded against by allowing frequent short intervals of rest, which were diminished as the activity period progressed. The increased metabolism of the period may be due in part to the development of more muscle tissue, but possibly more to the difference in metabolic processes which occur in a muscle made physiologically fit by exercise from one reduced to a state of fatigue by inactivity. Bainbridge (1919) maintains that the speeding up of the metabolic process, which occurs during exercise, involves the more rapid utilization of reserve nutrient material and probably also the more complete oxidation of these materials. In this way exercise decreases reserve material in the cells, and may accelerate the elimination of waste metabolites. After the exercise is over, the metabolic activity is, at least in part, diverted to repairing or building up tissues. Within limits, functional activity leads to repair processes or hypertrophy, a condition akin to

growth which is associated with a high metabolic rate. Disuse leads to atrophy as occurs in old age, and in man is accompanied by a low metabolic rate.

During the 9th week (January 6, 1926), after reinstatement of the daily muscular exercise, the dog was lost by accident. Autopsy revealed a very fat animal with no gross pathology. Histological studies of the thyroids

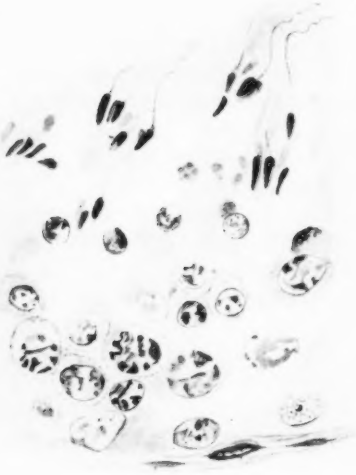

Fig. 1

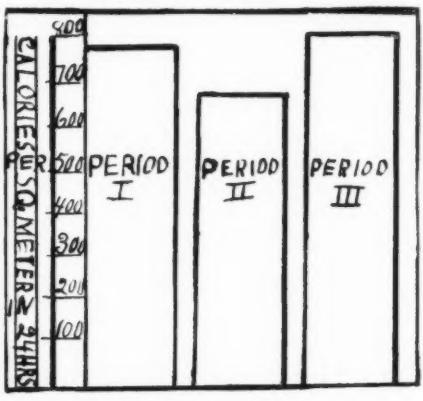

Fig. 2

Fig. 1. Portion of a cross section of a seminiferous tubule from dog II, illustrating the various stages of spermatogenesis after 22 months of inactivity followed by 9 weeks of daily indoor exercising (treadmill and liberty to run in a large room), camera lucida: 8×2.

Fig 2. The basal metabolism of dog II as influenced by changes in physical activity. Comparisons are based on an average of 10 daily 15 minute metabolism tests: period I (July 29, 1921, to January 1, 1924) standard amount of laboratory activity; period II (January 1, 1924 to November 1, 1925), rigorously restricted activity. Period III (November 1, 1925, to January 6, 1926), reinstatement of daily indoor exercise.

and testes, glands having special significance in metabolic processes, were made. The follicular epithelial cells of the thyroids were the high columnar type characteristic of glands in a normal state of functional activity. Figure 1 presents a camera lucida drawing of the testes showing spermatogenesis in all stages in active process. This is of special significance inasmuch as Oslund (1924) and others find that laboratory confinement in dogs tends to cause atrophy of the tubular epithelium. In the dog after

22 months of closest confinement followed by 9 weeks of daily exercising, in moderate amounts, the testes present the normal histological picture. Either degeneration due to confinement did not occur, or some process generated by the increased metabolism of the last period stimulated the seminiferous tubules to normal activity. Confirmatory experiments with histological studies of one testis at the end of the period of confinement and the other after reinstatement of daily exercising should be made.

In the case of dog IX no metabolism determinations were made before the period of inactivity, although the dog had been in this laboratory under dietary management for more than two years. The muscular exercise was not quite so rigorously restricted as is described for dog II. Exercising, after the inactivity, was encouraged by daily allowing out-of-door freedom for 2 hours, and 2 hours of indoor play with other dogs. After 5 months (May 11, 1926 to October 13, 1926) the metabolism increased 23 per cent. This is a greater increase than occurred in dog II as a result of indoor activity but the period of daily exercising for dog IX is much longer. Lusk (1913) reports a similar change in the metabolism of a dog after spending the summer months in the country. Steinhaus (1926) found (preliminary report) no increase in the basal metabolism of his dogs after several weeks of daily exercising (treadmill). In a personal communication, this author states that the conditions of his experiments differ from those of this report, in that the exercise was strenuous, and the protein content, as well as the total caloric value of the dietary, was much lower than that received by the dogs of our experiments.

Dog V was never closely confined to a small cage. During the first $2\frac{1}{2}$ years (April 1, 1923 to October 4, 1925) of laboratory life it was allowed the amount of activity described for period 1. Following this, October 4, 1925 to February 22, 1926, it enjoyed unrestrained liberty to run about the college campus and laboratory corridors. As a result of this the metabolism increased 10 per cent.

SUMMARY

1. The basal metabolism of dogs, on a high caloric diet, is decreased by restrictions of physical activity, and increased by daily exercise, short of that causing exhaustion.

2. The basal metabolism of 4 fully grown adult dogs, housed in the laboratory from 2 to 12 years, shows no decline as a result of the advanced age, when living under conditions of moderate amounts of daily exercise and a high protein diet.

3. The testes and thyroids of a dog after 22 months of rigorously restricted physical activity followed by $9\frac{1}{2}$ weeks of indoor exercising presents the normal histological picture.

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THE QUANTITY OF MILK PRESENT IN THE UDDER OF THE COW AT MILKING TIME

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On the basis of the literature of milk secretion and study of the physiology of the process in the cow in this laboratory we have tentatively adopted the theory as in part follows. No general attempt is made at this time to justify the theory—it is used rather by way of introducing the occasion for the particular investigation reported in this paper.

The peculiar constituents of the milk (lactose, casein, etc.) are elaborated within the milk secreting cells and accumulate therein together with the common constituents (water, etc.) creating a tension which ruptures the free ends of the cells, whence the milk fills and passes out of the alveoli into the system of conjoining and enlarging ducts and reservoirs, leading to the nipple orifice. The process proceeds continuously (not necessarily at uniform rate) between milkings. At milking time the full quantity of milk obtained upon milking has accumulated and is present as such in the udder. The smaller part is present in the milk cisterns and communicating larger (non-capillary) ducts, and is readily subject to removal by the physical forces employed in milking. The larger part of the milk is present in the finer (capillary) ducts or still within the secretory cells, and is not subject to removal by the physical forces of milking as employed on the teats alone until it has been expelled by other means into the cavities of the teats. This expulsion is accomplished by a contractile mechanism of the lactal[1] system which is set in action by a nervous reflex initiated by the stimulus of milking. Discharge of milk is thus enormously accelerated by the stimulus of milking; but milk secretion proper, that is, the chemical elaboration of milk, is not directly accelerated by the stimulus of milking. All this to be regarded as a working hypothesis.

The presence of milk in the udder of the heavily lactating cow at milking time is self-evident upon observation. The udder is greatly distended as compared with its condition after milking. Occasionally, in cases of injury or weak sphincters, milk leaks more or less continuously from the

[1] The term is used to designate the system of gland cells, ducts and reservoirs by means of which the milk is elaborated and passed to the nipple, in short, the milk secreting, storing and discharging organization of the udder.

teats between milkings. Upon slaughter milk exudes freely from a gash in the udder. The whole udder seems to be permeated with milk, and the slightest cut at any place in the glandular tissue of the dead animal immediately turns milky, even as the slightest cut in the flesh of the living animal turns bloody.

The presence of *some* milk in the udder at milking time must be admitted; there is a question as to whether *all* the milk is so present. There is a more or less widespread popular opinion that only a small fraction of the milk obtained upon milking is present as such at the beginning of milking. It is held that the larger part is secreted during the milking process, which occupies five to ten minutes. The following quotation from a recent college textbook (Judkins, 1924) illustrates the notion.

> The udder contains only a small amount of milk, usually between a pint and a quart, when one starts milking. This is found in the four milk cisterns. The enlargement of the udder which occurs before milking is doubtless due to the storing up of the ingredients out of which milk is to be made. . . . After the first milk is drawn, the cow, by nervous tension, tightens up the muscles located at the points where the ducts branch off, and simply stops making milk until she gets ready to do so. When that time comes, the gland lobules and their contents, in some mysterious way, put the stored ingredients together into milk, which trickles down through the ducts to the cistern; thence it passes to the teat canal, and the milker squeezes it out. For the most part, therefore, milk is really made during the milking process. A cow killed just before milking time will be found to have no milk in the udder except that present in the milk cisterns.

The quotation is given as representing more or less accurately the opinion of a considerable body of dairy people. In face of the most elementary observation some of the statements need revision. The assumption of "stored ingredients" or mother substances, and an enormously accelerated rate of milk secretion during the act of milking, makes any understanding of the process all the more difficult. Such assumption should be avoided until we are forced to it by the failure of simpler explanation. However, the only critical experimental evidence we have bearing on the matter has been interpreted by the experimenters as necessitating such assumption.

Maxwell and Rothera (1915), working with nursing cats, estimated the amount of milk yielded to the kittens and finally the amount of milk present in the glands at nursing time by killing the mother and analyzing the dissected glands for lactose. They concluded that about 40 per cent of the milk normally yielded to the kittens was secreted during the time of nursing and under the stimulus of the nursing. That is to say, there was that degree of shortage in the lactose found on post-mortem analysis.

It is apparent that the work of Maxwell and Rothera raises an important issue in connection with the physiology of milk secretion, nursing and

milking. Their interpretation of their results does not support the working hypothesis as set out above. We have thought it desirable, therefore, to conduct a similar investigation on cows, utilizing the principle that the milk present in the udder may be distinguished quantitatively from the other tissues of the organ by chemical determination of the lactose content. During the past two years three cows in the University herd have become available for such an investigation, due to the fact that they reacted to the tuberculin test and were therefore condemned to slaughter.[2]

Experimental procedure and results. Cow 25 was the first subject. She was fed a controlled ration and thoroughly hand milked at twelve-hour intervals by a skilled milker. The right and left halves of the udder were milked into separate containers. When the milk yield had reached a satisfactory regularity under the controlled conditions samples of each milking for each half of the udder were taken and analyzed for lactose. Finally, at the close of a regular milking period the cow was killed unmilked. Care was taken to hold the time element as constant as possible and it was held throughout within two or three minutes of the exact schedule. The killing was done by an experienced butcher after the usual fashion of his art. At the scheduled time the cow was felled unconscious by a blow on the head and quickly bled to death by sticking the carotid. As soon as bleeding ceased the udder was amputated, split at the median septum, and the halves subjected to analysis.

The left half was plunged into a bath of boiling water and boiling was continued for fifteen minutes. The intention here was to forestall any enzymic destruction of lactose.

The right half after removal of the hide was cut into chunks suitable for passage through a food grinder. The pieces were squeezed in the hands to remove some of the fluid. The milky fluid exuding by cutting and squeezing was only slightly colored with blood, indicating almost complete draining of the blood vessels by the method of slaughter.

After grinding, the tissue was washed with distilled water and strained. The process was twice repeated on the residue, all the washings being collected together with the milky exudate above. One to two liters of water were used at each washing. A small amount of formalin was used as a preservative. The final residue was dried on the water bath over night and reground for analysis. A sample of the dried material was weighed into a 500 ml. flask and extracted by boiling with 150 ml. of distilled water for ten to fifteen minutes. The boiling was accompanied by considerable frothing. The procedure after boiling was the same as with milk.

[2] Post-mortem inspection revealed very slight tubercular lesions in one or two lymph glands in each case. Aside from the technicality of having given a positive reaction to the tuberculin test the cows would all be classed as perfectly healthy individuals.

The left half of the udder, after boiling, was treated in a similar manner as followed with the right half. No preservative was used. In addition, one liter of the milky exudate and washings from the sliced and ground udder was treated with yeast to destroy any glucose present from blood and lymph and re-analyzed after eight hours.

The analyses for lactose were carried out by two methods, for the reason that we did not feel sure as to the most satisfactory method of determining

TABLE 1

Milk and lactose yield by milking and slaughter

Cow 25. Breed, grade Holstein. Age, 4 years. Weight, 990 pounds. In milk, 8 months. Left half of udder, boiled after amputation

DATE	NATURE AND SOURCE OF MATERIAL	WEIGHT OF MATERIAL	LACTOSE CONTENT OF MATERIAL			
			Copper method		Optical method	
1925		*grams*	*per cent*	*grams*	*per cent*	*grams*
August 14 a.m.	Milk by normal hand milking	2132	4.50	95.94	4.84	103.19
p.m.		2268	4.51	102.79	4.81	109.09
August 15 a.m.		2359	4.54	107.10	4.82	113.70
p.m.		2449	4.54	111.18	4.81	117.80
August 16 a.m.		2449	4.55	111.43	4.82	118.04
p.m.		2359	4.64	109.46	4.81	113.47
August 17 a.m.	Milky exudate and filtrate by slicing, grinding and washing udder*	8482	1.20	101.78	1.22	103.48
	Dried residue from ground and washed udder	442	1.48	6.54		6.65†
	Equivalent 4.82 per cent milk by summation (optical)	2286			4.82	110.13
	Equivalent 4.55 per cent milk by summation (copper)	2382	4.55	108.32		
August 14–16	Average milk by six normal milkings above	2336	4.55‡	106.23	4.82‡	112.55

* A test for the presence of blood sugar in this liquid was made by taking one liter of same, inoculating heavily with yeast and holding at slightly above room temperature for 8 hours. At the end of that time polariscopic reading indicated a slight increase, to 1.23, in terms of the lactose percentage of the table. There was no preservative in this sample.

† It did not appear feasible to carry out a polariscopic determination on this material because of uncertainty as to reliable procedure. The figure is an estimate based on the copper determinations in such manner as to preserve the ratio of the results of the two methods up to this point.

‡ Weighted by the amount of milk. Additional decimals have been used in the computations involving this factor.

lactose in the udder tissue. The methods were the Soxhlet-Wein copper reduction method of the Association of Official Agricultural Chemists (1925) using sodium thiosulphate, and the optical method of the same authority, using mercuric nitrate. Duplicate determinations were made in all cases. The duplicates agreed closely throughout, and the mean values only are here reported.

TABLE 2

Milk and lactose yield by milking and slaughter

Cow 25. Right half of udder, not heated

DATE	NATURE AND SOURCE OF MATERIAL	WEIGHT OF MATERIAL	LACTOSE CONTENT OF MATERIAL			
			Copper method		Optical method	
1925		*grams*	*per cent*	*grams*	*per cent*	*grams*
August 14 a.m.	Milk by normal hand milking	2087	4.50	93.92	4.84	101.01
p.m.		2268	4.51	102.29	4.80	108.86
August 15 a.m.		2313	4.54	105.01	4.81	111.26
p.m.		2404	4.52	108.66	4.80	115.39
August 16 a.m.		2404	4.54	109.14	4.82	115.87
p.m.		2359	4.62	108.99	4.81	113.47
August 17 a.m.	Milky exudate and filtrate by slicing, grinding and washing udder	6277	1.54	96.67	1.58	99.18
	Dried residue from ground and washed udder	290	2.06	5.97		6.13*
	Equivalent 4.81 per cent milk by summation (optical)	2188			4.81	105.31
	Equivalent 4.54 per cent milk by summation (copper)	2261	4.54	102.64		
August 14–16	Average milk by six normal milkings above	2306	4.54†	104.67	4.81†	110.98

* It did not appear feasible to carry out a polariscopic determination on this material because of uncertainty as to reliable procedure. The figure is an estimate based on the copper determinations in such manner as to preserve the ratio of the results of the two methods up to this point.

† Weighted by the amount of milk. Additional decimals have been used in the computations involving this factor.

The milk weights by normal hand milking were determined to the closest tenth pound by a tested and accurate spring balance. For simplicity in the tabular presentation the weights have been converted to the metric unit.

The results for the left and right halves of the udder are given in tables 1 and 2 respectively.

Cow 741 was the second subject. The udder was treated as a whole and essentially the same procedure followed as above outlined for the right half (not boiled), except that immediately after amputation the udder was suspended in a somewhat normal position and milked by hand

TABLE 3

Milk and lactose yield by milking and slaughter

Cow 741. Breed, Guernsey-Holstein (F_2 Hybrid). Age, 4 years. Weight, 950 pounds. In milk, 3 months. Weight of udder as amputated, 15,024 grams

DATE	NATURE AND SOURCE OF MATERIAL	WEIGHT OF MATERIAL	LACTOSE CONTENT OF MATERIAL			
			Copper method		Optical method	
1926		*grams*	*per cent*	*grams*	*per cent*	*grams*
October 21 p.m.	Milk by normal milking	7122	4.58	326.19	5.04	358.95
October 22 a.m.		7076	4.64	328.33	5.16	365.12
p.m.		7167	4.59	328.97	4.96	355.48
October 23 a.m.		7212	4.66	336.08	5.04	363.48
p.m.		7076	4.57	323.37	5.14	363.71
October 24 a.m.		7167	4.65	333.27	5.13	367.67
p.m.		6985	4.58	319.91	5.04	352.04
October 25 a.m.	Milk by hand milking of amputated udder	2488	4.66	115.94	5.06	125.89
	Milky exudate by slicing udder	2255	3.58	80.73	3.92	88.40
	Watery filtrate by washing ground udder	10,170*	0.83	84.41	0.98	99.67
	Dried residue from ground and washed udder	1110*	3.07	34.08		38.07†
	Equivalent 5.07 per cent milk by summation (optical)	6940			5.07	352.03
	Equivalent 4.61 per cent milk by summation (copper)	6836	4.61	315.16		
Ocotober 21–24	Average milk by seven normal milkings above	7115	4.61‡	328.02	5.07‡	360.92

* The quantity actually worked with was one-fifth this amount.

† It did not appear feasible to carry out a polariscopic determination on this material because of uncertainty as to reliable procedure. The figure is an estimate based on the copper determination in such manner as to preserve the ratio of the results of the two methods up to this point.

‡ Weighted by the amount of milk. Additional decimals have been used in the computations involving this factor.

without any massaging of the udder as in the normal manner. Some milk was readily obtained but after a minute or two the milker com-

plained that the udder was "not milking naturally."[3] The results are given in table 3.

With the third subject, cow 2, the optical method of analysis was omitted but otherwise the same procedure as with no. 741 was followed with one exception in technique which seems to be of much importance. The chunks of udder, after draining of milk, were passed through a power grinder with a finer plate and better knives than the one used previously, and this served to reduce the tissue to a very fine pulp, much finer than we had obtained in the other cases. This pulp was of such a nature that it could be thoroughly mixed by pouring from container to container and the mixed material accurately sampled in small quantity. A sample of about 85 grams was weighed, using a weighing tube, directly into a 500 ml. flask, and about 300 ml. of water added. The copper sulphate precipitant was then added, the whole heated to boiling for a few minutes, cooled and proceeded with as for milk. Washing and straining were thus dispensed with. As a check on the previous procedure a portion of the udder pulp was dried on the water bath, ground and analyzed.

A portion of the milky exudate from the sliced udder was treated with 0.05 per cent formalin, allowed to stand for twenty-four hours and the lactose content then redetermined. This milky exudate appears to have been considerably contaminated with lymph and cell juices judging by its low percentage lactose content (table 4) and it was thought a lactase might be present and detectable in the fluid.

The results for no. 2 are given in table 4.

Discussion. It is apparent from the experimental procedure as described, that we are depending upon a lactose determination for the quantitative estimation of the milk present in the udder of the cow at the time of milking. We are dealing with comparatively large quantities of lactose and the results indicate that there is practically no danger of an over-estimation of lactose due to the presence of sugars from other sources than milk. The advocate of mother substances may argue that what has been found may not be altogether lactose but also the mother substance of lactose. If so the burden of proof would seem to rest on such advocate. At least it seems to us rational to assume that the chemical processes have distinguished lactose and that a certain quantity of lactose represents quite definitely a certain quantity of milk.

It appears that no special precautions need be taken against loss of lactose through the action of an enzyme. Bradley (1913) tried and failed to demonstrate the presence of lactase in the mammary gland and if present its action is apparently feeble. The lower lactose recovery in the unheated half of the udder of no. 25 (table 2) as compared with the

[3] How could it milk naturally, if natural milking involves the functioning of a nervous reflex and a contractile mechanism?

boiled half (table 1) might be interpreted to mean that some lactose had been destroyed by enzyme action in the unboiled half during the process of preparation, which occupied several hours. In view of the later results, indicating incomplete recovery of lactose by the method used in this case, and in view of the small difference found, such an interpretation appears hardly justifiable.

TABLE 4

Milk and lactose yield by milking and slaughter

Cow 2. Breed, grade Holstein. Age, 5 years. Weight, 1225 pounds. In milk, 7 months. Weight of udder as amputated, 17,102 grams

DATE	NATURE AND SOURCE OF MATERIAL	WEIGHT OF MATERIAL	LACTOSE CONTENT OF MATERIAL	
			Copper method	
1926		*grams*	*per cent*	*grams*
October 29 a.m.	Milk by normal hand milking	7076	4.75	336.11
p.m.		7076	4.56	322.67
October 30 a.m.		7258	4.77	346.21
p.m.		6985	4.69	327.60
October 31 a.m.		7303	4.64	338.86
p.m.		7348	4.56	335.07
November 1 a.m.	Milk by hand milking of amputated udder	1593	4.70	74.87
	Milky exudate by slicing udder*	2744	3.72	102.08
	Wet, finely ground udder tissue†	10,426	2.10	218.95
	Equivalent 4.66 per cent milk by summation (copper)	8493	4.66	395.90
October 29–31	Average milk by six normal milkings above	7174	4.66‡	334.42

* A sample of this liquid after standing 24 hours at room temperature showed 3.71 per cent lactose by the copper method, and thus no evidence of any action of a lactase.

† After the determinations on this material had been made a sample (1618 grams) which had been retained was subjected to the drying process and analysis as used in the previous trials. The dried sample weighed 298 grams and contained 5.44 per cent lactose. This is equivalent to 1.00 per cent lactose in the wet material (as compared with 2.10 per cent found by direct determination without drying).

‡ Weighted by the amount of milk. Additional decimals have been used in the computations involving this factor.

On the basis of the lactose yield at previous milkings, using the results by the copper method as the more reliable, and dealing with the udder as a whole, the results for the three cows show a lactose recovery upon slaughter as follows:

Cow 25, 100 per cent
Cow 741, 96 per cent
Cow 2, 118 per cent

The much larger recovery in the case of no. 2 we attribute to more complete extraction of the lactose present in the gland pulp, by dealing with it directly instead of first washing and drying. The washed pulp is very difficult to filter or strain. It is possible that lactose remaining in the residue was partly destroyed by caramelization in the drying process, and extraction of the dried residue may have been incomplete. In any case, direct dealing with the wet pulp is more productive as well as very much easier, as compared with the method of washing and filtering.

In case of no. 741, washing of the ground tissue, because of the tedious nature of the process, was not carried out as thoroughly as with no. 25, and we feel confident that more than 100 per cent of the average lactose yield of the preceding milkings was present in the udder at slaughter for these two cows as well as for no. 2. *We infer accordingly that at the time of milking there is present in the udder of the cow a quantity of milk in excess of that obtained upon milking.*

The results indicate further that, very roughly, one-fourth of this milk may be obtained by milking the amputated udder, another fourth by drainage from the sliced tissues, while the remaining half is held very tenaciously, presumably by capillary forces or within the unruptured milk secreting cells. Clearly some sort of milk-discharging mechanism may be regarded as a beneficial, if not an actually necessary, adjunct of the mammary apparatus.

The working hypothesis outlined at the start we regard as in keeping with the present experimental results, so far as they go.

To determine by the present general line of procedure, whether or not the rate of milk secretion is directly accelerated by the stimulus of milking, it would be necessary to know the amount of lactose present in the gland, at the close of milking as well as the amount of lactose secured upon milking, the sum of these two amounts to be used in comparison with the amount of lactose present in the unmilked udder at milking time. Unfortunately, the amount of lactose present in the gland at the close of milking cannot be directly determined without destroying the organ and thus preventing completion of the experiment with that particular animal. Indirect approach must therefore be used and this angle of the general problem we hope to attack as opportunity offers in the future.

The present experimental results and interpretation it will be apparent are in conflict with those of Maxwell and Rothera. We are loath to think that the process of milk secretion is fundamentally different in the cat than it is in the cow. On this account we have been inclined to review their paper somewhat critically. There seems to be some more or less serious chance for error in their procedure. The milk yield of their cats was estimated by separating the kittens from the mother for a few hours, then returning the kittens to nurse and determining the change in weight

of mother and kittens by nursing. The kittens were then left with the mother until the following day when the procedure was repeated. On the third day the kittens were again separated, but at nursing time the cat was killed and the glands analyzed. The investigators seem to have been somewhat careless of the time element judging by the one protocol which gives this factor. In this case, the milk yield was determined by nursing after $6\frac{1}{2}$ hours' separation and lactose content of the glands by killing after 6 hours' separation. This difference in time would seem to introduce the possibility of an 8 per cent error. It would, furthermore, have been better practice to have kept the kittens separated continuously except at regular nursing intervals, since with their procedure there is no way of knowing with any certainty at what time preceding separation the kittens may have last nursed the mother. The time of nursing rather than the time of separation is the important factor.

A still more serious fault of their procedure is found in the failure to determine the percentage lactose content of the milk of the individual mother cats. It is obvious, that this factor is an essential in the estimate of the amount of lactose. They have used the factor 5.07 per cent lactose without any statement as to where this figure comes from. The literature on the lactose content of cat's milk is very meager.

Folin, Denis and Minot (1919) give analyses of nineteen individual samples of the milk of three cats and the analyses show a range of 2.3 to 4.0 per cent lactose. The average of twenty-six samples from four cats is given as 3.4 per cent lactose.

These analyses constitute the only direct report on the lactose content of cat's milk which we have been able to find. They show the weakness of the method of Maxwell and Rothera in assuming 5.07 per cent lactose. Use of the factor 3.4 instead of 5.07 would lead to an entirely different interpretation of their results. This leads us to believe that a proper interpretation of the results of Maxwell and Rothera with the cat would be entirely in keeping with the present interpretation of our results with the cow.

SUMMARY

The quantity of milk present in the udder at milking time was estimated by milking at regular twelve-hour intervals, determining the weight and lactose content of the milk, and finally killing the cow (unmilked) at a regular milking time. The milk and tissues of the amputated udder were analyzed for lactose. On the basis of the average lactose yield at the regular milkings the percentage recovery upon slaughter for the three cows used was 100, 96 and 118. It is believed that the last result is the most reliable because of improved technique of udder analysis. It is inferred, therefore, that there is present in the udder at milking time an

amount of milk in excess of the amount secured upon milking. Very roughly, one-fourth of the milk is obtainable by milking the amputated udder, another fourth by drainage from the sliced udder, while the remaining half is tenaciously held even in the finely ground tissues.

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THE SYNTHESIS OF VITAMIN E BY PLANTS GROWN IN CULTURE SOLUTIONS

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Few researches have been attempted, the aim of which has been an understanding of the conditions attending the synthesis of vitamins by plants. Entirely apart from the possible practical value of a comprehension of the circumstances under which vitamins are abundantly or scantily produced in plant tissues, theoretic importance attaches to the subject. Even if the plant itself has no nutritive dependence upon organic "traces," which, as is well known, Bottomley has called the auximones, the synthesis of vitamins may have such dependence. The use of pure inorganic nutrient solutions would appear capable of settling this point. We were aware of the fact that Coward and Drummond had already demonstrated the synthesis of vitamin A by plants grown in such cultures in sand. Our own interest was confined to an examination of whether vitamin E (which we have previously designated as "X") is synthesized under similar conditions. We have avoided sand, using merely a culture solution, the composition of which was as follows: expressed as parts per million—K 170, Ca 170, NO_3 700, PO_4 120, SO_4 200, Mg 50, Fe added as needed in the form of ferric tartrate. The plant employed was the Canadian field pea.

We have used etiolated as well as normal green plants and also plants which had been subjected to solutions of high salt (Na Cl) content, but the levels at which the plant tissues were fed were apparently too high to show possible differences in the vitamin content of the three types of plant. The etiolation only attained a partial absence of chlorophyll. In all cases the plants were fed to vigorous young female rats, normal in every respect save for the peculiar sterility disease which one of us has described as due to lack of vitamin E. The animals were reared on a basal diet of casein 18, corn starch 54, lard 15, milk fat 9, salts (185) 4, yeast .4-.6 gram daily. The existence of this disease was proven in the case of each individual by permitting a trial gestation to occur, a gestation which ended in a typical resorption. In all cases fertility resulted from conveying to such animals in addition to their basic pure food diet about 4 grams daily of the entire fresh pea seedling plant. The detailed histories follow.

TABLE 1

Showing the reproductive performance of five rats reared on the basic ration and, after a trial gestation on this diet, fed in addition plants of the Canadian field pea seedling

CURATIVE FOODSTUFF	DESIGNATION OF RAT	GESTATION ON THE BASIC RATION	TIME OF MODIFICATION OF THE BASIC RATION WITH REFERENCE TO THE TEST GESTATION	DATE OF POSITIVE MATING	DATE OF FINDING OF PLACENTAL SIGN (R.B.C.)	DATE OF ISOLATION	DATE OF LITTERING	NUMBER OF YOUNG BORN
4 grams of fresh green pea seedling daily	W 8937	Litter 2 (dead)	9 days before mating	2-16-23	3-1-23	3-7-23	3-12-23	2
			Dosage stopped two days before littering; animal mated again	4-8-23	4-22-23	4-27-23	5-1-23	3
	W 9242	Resorption	Day of mating	2-7-23	2-21-23	2-26-23	2-28-23	7
			Dosage stopped 9 days after littering; animal mated again	3-8-23	3-22-23	3-27-23	3-30-23	3
4 grams of fresh etiolated pea seedling daily	B 9178	Resorption	2 days before mating	2-9-23	2-23-23	2-28-23	3-3-23	3
	W 9180	Resorption	3 days before mating	2-10-23	2-23-23	3-1-23	3-4-23	3
4 grams of fresh alkali pea seedling daily	G 9243	Resorption	9 days before mating	1-30-23			2-21-23	5

We regard these results as indicating that the green plant synthesizes vitamin E from inorganic sources, admitting freely the contention that bacterial contamination of the nutrient solution was not avoided and that there would be the desirability of such a control.

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THE QUESTION OF LOCALIZING ACTION CURRENTS IN MUSCLE BY NEEDLE ELECTRODES

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The interpretation of the electromyogram has been a matter of considerable controversy and not without confusion. The typical electromyogram of a skeletal muscle during sustained contraction of reflex or voluntary origin shows a highly irregular sequence of excursions revealing action currents almost devoid of any definite rhythm. In spite of this irregularity there often appears to be a dominant rhythm. In the case of the flexor muscles of the forearm in man some observers have found a fairly definite rhythm of approximately 50 per second (Piper, 1912; Forbes and Rappleye, 1917). Other observers have failed to detect this. In all electromyograms, whether this dominant rhythm appears or not, there are many irregular excursions and small notches which make it impossible to state the true frequency with precision.

Perhaps the question which has been most discussed in connection with the electromyogram is whether such rhythm as appears can be taken as revealing a corresponding rhythm in the discharge of impulses from the motor neurones. Some authors contend that the frequency of motor nerve impulses can be read directly from the electromyogram (Piper, 1912; Cooper and Adrian, 1924; Adrian, 1925). Others have maintained that the nerve-impulse frequency is higher than the muscular responses are able to follow (Forbes and Rappleye, 1917; Forbes and Olmsted, 1925).

This question involves another more immediate question; namely, whether the observed frequency of excursions in the electromyogram can be taken as representing the rhythm of response in the individual muscle fiber or whether, being a composite picture of the responses of many fiber groups, independently innervated and responding out of phase with one another, it tells us nothing of the true rhythm of muscle response. Piper argued that the electromyogram revealed the frequency of response of the individual muscle fiber, since in maximal voluntary contraction the major oscillations, whose frequency he found to be about 50 per second, showed approximately the same size and time relations as the response to a maximal induction shock applied to the motor point of the same muscle. His argument appears to be valid in the case of those electromyograms

which reveal this property. On the other hand, even those electromyograms which show large, regular excursions also show intervals characterized by small, irregular waves in which no definite rhythm can be detected and in which it is reasonable to suppose that the individual fiber groups are responding out of phase with each other.[1] In a large majority of reflex electromyograms obtained from animals the largest excursion is small compared with that evoked by a maximal stimulus, that is, one which involves all the fibers simultaneously. Therefore in such cases Piper's reason for supposing that the fibers are mostly responding in unison does not apply. Cooper and Adrian (1924), working with the flexion reflex in the spinal cat, found indirect evidence which led them to suppose that the apparent rhythm in their electromyograms represented the characteristic rhythm of response in the individual muscle fiber and also reflected the actual rhythm of discharge of motor nerve impulses. Forbes and Olmsted (1925) showed that their evidence on these points was equivocal; it could all be interpreted on the basis of a composite picture of muscle fibers responding out of phase with one another, and therefore telling nothing of the rhythm in the individual fiber, and furthermore furnishing no proof that any individual muscle fiber was able to respond with the actual frequency of the nerve impulses exciting it. Indeed Forbes and Olmsted found evidence that in the case of the crossed extension reflex, at least, the motor nerve impulse frequency, though probably irregular, was in general higher than any frequency appearing in the electromyogram of the muscle involved, and presumably higher than the muscle fibers were able to follow.

There seems to be a widespread belief that the source of confusion which may result from taking a composite picture of the responses of an entire muscle can be eliminated by using needle electrodes thrust into the substance of the muscle. Such electrodes, making contact with a limited number of muscle fibers, are supposed to lead off the action currents of those few fibers, or at most those in the immediate vicinity of the needles. This view is explicitly expressed by Bass and Trendelenburg (1921), who have reported experiments which they hold to substantiate this supposition. They speak of using needle electrodes to lead off from narrowly limited bundles of muscle fibers, and thus simplifying the action current picture. Dusser de Barenne and Brevée (1926) and Golla and Hettwer (1924) have assumed that the fiber groups led off are closely limited in the case of needle electrodes and they have based far-reaching conclusions on this assumption. The arguments are of such theoretical importance that they should be considered in detail.

[1] It is well established that each motor neurone innervates several muscle fibers; all of the muscle fibers in a group thus innervated by a single nerve fiber, must normally respond approximately in unison.

Golla and Hettwer (1924), dealing with the problem of reciprocal innervation of antagonistic muscles in the human subject, used needle electrodes to isolate the action currents of a muscle from those of its antagonist. The two strings of a double string galvanometer were connected with two pairs of needle electrodes, and each pair was inserted into one muscle of an antagonistic couple, e.g., biceps and triceps. Observing with this method that a voluntary movement was attended by oscillations in the electromyograms of both muscles, they inferred that both were contracting at the same time. The divergence of this inference from Sherrington's principle of reciprocal innervation, according to which contraction of one muscle is normally facilitated by inhibition of its antagonist, is a matter of importance to physiology and neurology. The conclusion of Golla and Hettwer rests on the unproved assumption that the needle electrodes led off only the action currents of the muscle in which they were inserted. If the action currents of the antagonist, lying on the other side of the limb, could contribute materially to the electromyogram of the muscle supposedly thus isolated, the conclusions drawn from these experiments would be invalidated.

Dusser de Barenne and Brevée (1926) studied the electromyogram of the decapitate cat, evoked by mechanical pressure applied to the dorsal surface of the spinal cord, and the change induced by the application of novocaine directly to the cord. Before narcosis the mechanical stimulus evoked a typical electromyogram with irregular excursions whose average frequency lay between 100 and 200 per second. When the cord was narcotised the excursions became gradually smaller, and finally a stage was reached in which there were small oscillations of a frequency between 50 and 70 per second and far more regular than are ever seen in an ordinary electromyogram; the increase in regularity was indeed striking. This interesting observation was taken as evidence that the observed frequency in the electromyogram is a true image of the discharge of impulses from the central nervous system. An essential step in the argument is the assumption that the muscle fibers which contribute to the electromyogram are responding in unison. This assumption was reinforced by the following observations of Bass and Trendelenburg. These authors applied two pairs of needle electrodes to a muscle and connected each pair with one of the two strings of a double string galvanometer. When the fiber bundles with which these two pairs of leads made contact were not more than two centimeters apart transversely, the electromyograms obtained with the two strings simultaneously from the same contraction showed consistent synchronism of practically all the oscillations in the two records.

This observation is open to two interpretations. 1. Assuming that each pair of needle electrodes only leads off the action current of the fibers in contact or almost in contact with them, the synchronism of oscillation

in the two electromyograms would prove that, although their rhythm is not perfectly regular, the muscle fibers in a transverse area of muscle as wide as two centimeters respond always in unison. 2. The other interpretation is that the fibers contributing to the electromyogram from each pair of leads covers so large a transverse area that the two areas overlap extensively, indeed, that the two pairs of electrodes lie near the center of a common sphere of influence. This view carries no implication as to synchronism among the fiber groups. Judging from the irregularity of the record, they are presumably responding in a more or less haphazard way and quite out of phase with each other, but most, if not all of the fibers lying between the two pairs of electrodes, and perhaps more too, contribute to both electromyograms; hence the apparent identity of the rhythm in the two records.

Bass and Trendelenburg adopt the former interpretation and in support of it they adduce the following observations. With both pairs of electrodes in place and connected with the two strings in the galvanometer, an external electromotive force amounting to as much as 100 millivolts applied to one pair of electrodes caused no visible excursion of the string connected with the other pair of electrodes. From this it is inferred that the action currents arising in the fibers close to one pair of electrodes could not induce an appreciable excursion in the galvanometer connected with the other pair. That this conclusion is not warranted follows from the observations of Davis and Brunswick (1926) discussed by Davis (1926). The experimental evidence gives reason for concluding that a large part of the action current is short-circuited by the surrounding fluid and connective tissue. Davis pointed out that "we have as yet but little knowledge of the fraction of the total bioelectric potential which reaches our recording instrument." In like manner the externally applied electromotive force is probably to a large extent shunted from the active fibers by the connective tissue and fascia surrounding the muscle. Therefore this observation of Bass and Trendelenburg cannot be considered strictly quantitative and furnishes no proof of the extent to which a recording circuit may be shielded by the intervening tissues from the electromotive force set up by muscle fibers responding at a distance.

Fortunately, it is an easy matter to put this question to experimental test. If, when needle electrodes are used as leads, only those fibers lying close to them produce the excursions in the electromyogram, and remote portions of the muscle fail to influence the record, surely the action currents of a muscle on the opposite side of the limb should fail to evoke any excursions whatever in the record. It is a simple matter to stimulate separately two antagonistic muscles on the opposite sides of a limb by means of their motor nerves and to see whether the action currents of one

muscle can be recorded through needle electrodes applied to an antagonistic muscle. If they can, the statement that only those fibers lying close to the electrodes play a part in the electromyogram is clearly unwarranted, and the alleged localization of the recorded response in those fibers closely adjacent to the electrodes is illusory.

METHOD. Cats were used in all experiments; in one case decerebrate, in the others, anesthetized with urethane. Decerebration was performed with the Sherrington guillotine under deep ether anesthesia. The urethane method seemed preferable since it is simpler and the nerve-muscle preparation functions normally in spite of this anesthetic. The muscles employed were the tibialis anticus and the gastrocnemius-plantaris-soleus group.

When the animal was anesthetized an incision was made in the thigh from hip to knee. The peroneal and popliteal (tibial) branches of the sciatic nerve were dissected apart centrally to the hip. They were then cut, and shielded stimulating electrodes applied to the peripheral portions of each. These electrodes were connected with a double-pole double-throw switch whereby the shocks of the inductorium could be applied to either nerve at will by merely closing the switch in the proper direction. The cathode on the break shock was always distal. A rigid clamp was applied to the femur, and the lower leg was steadied with another clamp.

The leading-off electrodes consisted of sharp silver needles coated with AgCl by electrolysis. They were thrust into the muscle substance to a distance of from 3 to 10 mm., small openings in the skin over the points of application being made with scissors to facilitate their insertion.

The procedure was to insert the electrodes into the tibialis anticus muscle, then to stimulate alternately the peroneal nerve, which innervates this muscle, and the popliteal, which innervates the antagonistic group, using in each case maximal break shocks. When an adequate series of observations had been recorded, the electrodes were inserted into the gastrocnemius muscle, and the procedure of alternately stimulating peroneal and popliteal nerves was repeated. In the case of each muscle we endeavored to place both leads distal to the nervous equator, in order to render the action currents simply diphasic (see Piper, 1912). The distance between the leads was so chosen that the resulting galvanometric excursion would be of a convenient size. It is possible to reduce the excursion by placing the leads so near together that the second phase of the action current will arrest the movement of the string before the disturbance at the first lead reaches a maximum. In some of our observations the excursions of the string were so large that its shadow left the film, but in the majority this was prevented by approximating the leads, tightening the string or reducing its magnification by means of an adjustable ocular. The distal lead in the case of each muscle was 20 to 40 mm. from the ankle; the distance between leads varied from 14 to 30 mm. No attempt was made to

render the contractions isometric or in any way restrain the resulting motion of the ankle-joint. Consequently contraction of the muscle caused a slight alteration in the electrode contact and therefore a change of base line in the galvanometric record. But this is of no consequence, since the mechanical shift was not appreciable until after the action current had appeared in the record, and for our purpose only the first phase of the action current is needed to compare the response of the muscle in which the electrodes are inserted with that of the antagonist. A subsequent alteration of the base line is irrelevant.

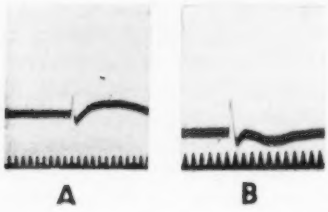

Fig. 1. Action currents of limb muscles led off through needle electrodes inserted in resting antagonists.

A, November 22, 1926. Leads in tibialis anticus muscle; maximal stimulus to popliteal nerve (innervating gastrocnemius). Stimulus approximately 38 Z units (Martin). String tension, 114 meters per amp. (see Forbes and Ray, 1923, This Journal, lxiv, 439). Magnification, 340.

B, November 29, 1926. Leads in gastrocnemius muscle; maximal stimulus to peroneal nerve (innervating tib. ant. muscle). Stimulus, 23 Z (approx.). String tension, 95 m. per amp. Magnification, 490. Time below; 1 d. v. = 0.01 sec.

To record the action currents we used a Hindle string galvanometer with a 1.5 mm. air gap and a gilded quartz string of 2.5μ diameter and 18,700 ohms resistance. As a source of illumination for the galvanometer, the arc lamp, hitherto used in this laboratory, was replaced by a 6-volt, 18-ampere tungsten ribbon light, operated from the power mains through a variable rheostat. This light, though less intense than the arc, is powerful enough to give satisfactory contrast on the film running at the maximum speed of the camera (35 cm. per second) with a magnification of 490 diameters. With slower speeds of film adequate contrast is obtainable with the current in the ribbon reduced to 17, or even 16 amperes. The great advantage of this light is its complete freedom from flicker, which is often a serious defect in the arc, leading even to an illusion of galvanometric deflections in the photographic record. The simplicity and convenience of operation, as well as the steadiness, render the ribbon light highly satisfactory.

The camera with which the action currents were photographically recorded has been described in a previous communication (Forbes and Thacher, 1920). It is so constructed that a large number of observations can be made in rapid succession. This made it possible to take enough records with varying strength of stimulus to show that the responses were maximal, and to alternate frequently between the stimulation of the muscle in which the electrodes were inserted and its antagonist. Since the com-

plete experiment as described above involves the use of only one leg, it is possible to repeat the entire experiment on the other leg of the same animal. In other words, one animal serves for two complete experiments. Three animals were used, and on them five complete experiments were performed; each experiment comprised a large number of alternating observations. The perfect uniformity of our results, as regards the main issue under investigation, in several hundred observations in the course of these five experiments, was such as to render further experimentation unnecessary.

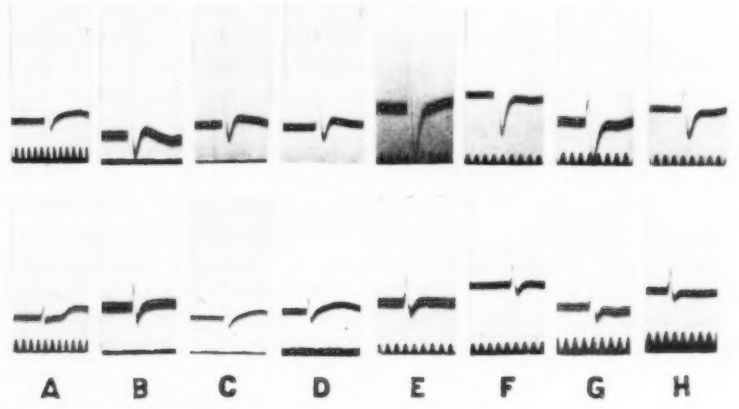

Fig. 2. Comparison of maximal action currents of tibialis anticus muscle (upper row) and of gastrocnemius group (lower row) led off in each case through needle electrodes inserted in tibialis anticus. The records in each vertically placed pair were taken in close succession without disturbing the leads.

A, November 15, 1926; *B*, *C* and *D*, November 22; *E H*, November 29. November 15 and 22, string tension, 114 m. per amp.; November 29, 95 m. per amp. Magnification in *A*, *C*, *D*, *F* and *H*, 340; in others 490. Stimulus in approximate Z units: *A*, upper, 4; lower, 6; *B*, both 12; *C*, upper, 6; lower, 12; *D*, both 23; *E*, upper, 6; lower, 12; *F*, upper, 12; lower, 23; *G* and *H*, both 23.

Results. The essential result of these experiments can be briefly stated. In every case, whether the leads were in the tibialis anticus muscle or in the gastrocnemius, the action current of the antagonistic muscle could be recorded with ease, and indeed registered a considerable excursion on the film. This fact is illustrated in figure 1. As was to be expected, the excursions were much smaller in the case of the antagonistic muscle than in the case of the muscle to which the electrodes were applied. But the response of the remote muscle was in every case so large as to be by no means negligible in comparison with that of the muscle directly led off. Typical records illustrating this point are shown in figures 2 and 3. In

the case of figure 2 the leads were in the tibialis anticus muscle, and the responses of this muscle were compared with those of the gastrocnemius group. In the case of figure 3 the leads were in the gastrocnemius muscle and the reverse comparison is made. In neither case were the ratios of excursions constant. When the electrodes were applied to the tibialis anticus the ratio of the excursion resulting from maximal response in the antagonistic group to that of the tibialis anticus itself varied from 1:2.6 to 1:7.3. The case of figure 2, *G* and *H*, in which the first phase was actually larger when the remote muscle was responding, is probably due to placing the leads on opposite sides of the nervous equator of the tibialis anticus muscle (see Piper, 1912, figs. 15 and 17). This was not included in the above summary of ratios, but is reproduced to show how grossly a remote muscle may confuse the record with the leads in certain positions. In the reverse experiment, i.e., with leads in the gastrocnemius, the corresponding ratios lay between 1:2.5 and 1:7.5.

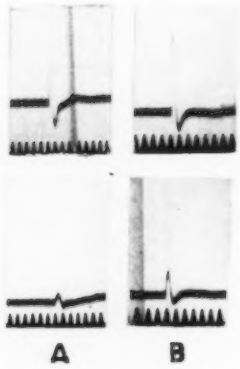

Fig. 3. Reverse of figure 2. Leads in gastrocnemius muscle. Upper row, stimulus to gastrocnemius group (popliteal nerve). Lower row, stimulus to tibialis anticus (peroneal nerve).

A, November 15. String tension, 114 m. per amp., magnification, 340. Stimulus in Z units; upper, 3; lower, 12.

B, November 29. String tension, 95 m. per amp., magnification, 340. Stimulus; upper, 38; lower, 23.

These results are not at all surprising, since there is no *a priori* reason for supposing that the action current of a group of muscle fibers a few millimeters or centimeters away from a pair of electrodes would fail to reach them with sufficient intensity to be recorded with a string galvanometer. Indeed it is a matter of common knowledge that the action current of the heart can be recorded through the hand, although this involves conduction through many centimeters of tissue (cf. Forbes and Miller, 1922, p. 124). It is clear that the insertion of needle electrodes into the substance of a muscle causes a relative increase in the part played by the fibers with which it comes most nearly in contact. But to suppose that thereby the influence of all other fibers in the limb is excluded is certainly not warranted. The difference between electrodes applied to the intact skin and needle electrodes thrust into the substance of the muscle may be likened to the difference between the sound of general conversation in a room full of people as heard by one standing outside of the room with the door closed and by one standing inside of the room. The listener outside of the room hears a muffled hum of conversation. Entering the room he hears the voices of

those nearest him far more clearly than he did when outside, but the voices of those on the opposite side of the room, instead of becoming inaudible have actually become louder, although relatively less loud than those nearest him.

DISCUSSION. The direct observation that the action currents of a muscle can readily be recorded through needle electrodes thrust into the substance of its antagonist on the opposite side of a limb, clearly shows that the work of Golla and Hettwer rests on an invalid foundation. Their records do not prove that the two antagonistic muscles were really contracting simultaneously, since they may have been simply recording action currents through both pairs of leads from one muscle or synergic group of muscles. It is clearly possible by voluntary effort to contract two antagonistic muscles simultaneously, and in many voluntary acts it is quite probable that this normally occurs. On the other hand, there is no doubt that the spinal reflexes normally involve reciprocal innervation, whereby one muscle is inhibited while its antagonist contracts, as was demonstrated by Sherrington. It is also probable that in the performance of many voluntary acts, a similar reciprocal innervation is employed.

Turning to the question of the extent to which remote fibers in the muscle to which the electrodes are applied contribute to the electromyogram, surely if a muscle on the opposite side of the limb can contribute appreciably, *a fortiori* the entire muscle to which the electrodes are applied must contribute appreciably. We are therefore in a position to decide between the two alternative explanations of the observations of Bass and Trendelenburg. Instead of assuming that all of the fibers between the two pairs of leads responded in the same irregular rhythm, that is, always in unison, we must conclude that the similarity of the records obtained with the two pairs of leads showed that each pair recorded the activity of a sphere of influence so large that the other pair lay fairly near its center; in other words, that the two spheres of influence overlapped extensively. There is therefore no reason to suppose that the fiber groups in a muscle as a whole respond in unison with one another.

This has an important bearing on the conclusions of Dusser de Barenne and Brevée. These authors, assuming that the electromyogram revealed the actual frequency of response in the individual muscle fiber, arrived at the further conclusion that this is a true image of the frequency of discharge of motor nerve impulses from the spinal cord. They took the irregular rhythm which appeared before they narcotized the spinal cord to be that imposed by afferent impulses, and the slower, more regular rhythm appearing in the later stages of narcosis, to be the natural frequency of discharge of impulses on direct mechanical stimulation of the motor neurones after the afferent source of excitation had been abolished. Since we are dealing with a composite picture of many fibers, presumably responding

out of phase with each other, we are not justified in any conclusion as to the frequency of response of the individual muscle fiber. The reasoning of Forbes and Olmsted, 1925 (pages 49 to 57) is in a general way applicable to these experiments, as to those of Cooper and Adrian. Such observations as those under discussion leave us still in the dark as to the true frequency of motor neurone discharge. The slow, regular rhythm observed by Dusser de Barenne and Brevée after narcosis of the spinal cord, is most interesting and may well be correlated with the refractory phase of some part of the reflex arc or motor neurone, presumably the last to succumb to narcosis under the conditions of their experiment. We should look on the spinal cord as a place in which extensive branching of afferent fibers causes a complicated sequence of motor nerve impulses, resulting in a confused and irregular picture when the action currents of an entire muscle are recorded. The effect observed under narcosis by Dusser de Barenne and Brevée may well be due to the cutting off of the afferent impulses which produce this irregular and haphazard discharge. Their cessation may leave some part of the synapse or motor neurone with a comparatively long refractory period, still free to be excited by the mechanical stimulus. A comparative uniformity of refractory phase in the mechanically excited structures would account for the regularity of the rhythm.

SUMMARY

1. A number of physiologists have assumed that when needle electrodes are used to lead off the action currents of a muscle, only those fibers which touch the electrodes, or at most those in close proximity to them, contribute appreciably to the resulting electromyogram. On this unproven assumption certain important conclusions concerning the central nervous system have been based.

2. In the present experiments on the tibialis anticus and gastrocnemius muscles of the cat, needle electrodes inserted in either one of these antagonistic muscles have been shown to lead off a considerable action current from the opposite muscle; i.e., when the muscle containing the leads was at rest and its antagonist alone was stimulated. The resulting excursions were, of course, smaller than those derived from the muscle containing the electrodes, but were large enough to show that conclusions based on the assumption that they are negligible are unwarranted.

3. On the assumption of the localizing property of needle electrodes Golla and Hettwer were led by their experiments to conclude that in voluntary activity antagonistic muscles normally contract simultaneously. On the same assumption Dusser de Barenne and Brevée were led to the conclusion that the electromyogram directly reveals the frequency of the motor nerve impulses discharged from the spinal cord. In view of the demonstration that the premise is invalid, the conclusions in both of these

researches rest on an inadequate foundation and cannot be considered established.

We wish to thank Miss L. H. Rice and Mr. J. H. Emerson for help in the experiments and in preparing the paper for publication.

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CHANGES IN THE CLOTTING TIME OF THE BLOOD OF CATS AS A RESULT OF EXERCISE[1]

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Cats were exercised in a treadmill as previously described (1). Blood was drawn as indicated in the table, during or at the end of the experiment.

The method employed for the determination of clotting time in our experiments was very simple. The ear (pinna) was shaved and thoroughly cleaned and then coated with paraffin oil. The marginal vein was punctured by a sharp triangular needle. Escaping blood was drawn up into a clean, oiled glass tube by decompression of a small rubber bulb at one end. The tube was about 40 mm. long and 3 mm. in diameter for half the length, the remainder being drawn out to capillary dimensions. Immediately after filling with blood, the bulb was slipped off so that a fine glass rod could be inserted from time to time. At intervals of thirty seconds or less the oiled rod was pulled through the blood to catch fibrin if present. The first appearance of fibrin was taken as the end point in our experiments. This gives the time required for the onset of clotting, which seemed to be more definite than some later stage. In doubtful instances a second sample of blood was tested. When it was thought that repeated bleedings from the same incision might be the cause of the reduced clotting time, a determination was made upon blood from the opposite ear. Twenty-one cats were studied. Thirteen showed a decrease, five an increase and three an increase followed by a decrease in coagulation time. The typical change therefore was decrease in coagulation time. The decrease might appear promptly or in some instances after some delay. Reduction in time often persisted for several hours (see cats 4, 6, 8, 11, 12, "Apr. 8," "Apr. 14," "Apr. 22"). There might be considerable variation in the coagulation time in the same cat when studied a few days apart although the direction of the change was the same (cat "Apr. 14").

The coagulation time was determined in one rabbit (wt. 3.290 kgm.). It was 120 seconds at the start, 285 seconds immediately after 287 meters of exercise and 135 seconds after 160 minutes of rest.

An important factor in the decreased clotting time is probably the increased output of epinephrin due to exercise because it has been shown that

[1] Aided by a grant from the Elizabeth Thompson Science Fund.

Changes in blood coagulation time of cats after exercise

CAT NUMBER	BEFORE	IMMEDIATELY AFTER		ONE HOUR OR MORE LATER	A FEW HOURS LATER	CHANGE
		575 m.	1150 m.			
1	90 sec.		110	80 sec. (2 hr.)	120 sec. 4 hr.)	Small increase
2	120		120	140 (3 hr.)	110 (7 hr.)	Small increase
3	105	140	165	95 (1 hr.)	110 (4 hr.)	Increase
4	200	140	165 (943m.)	130 (1 hr.)	110 (7 hr.)	Decrease
5	120	145	140	75 (2 hr.)	120 (5 hr.)	Increase, then decrease
6	105	105	70	80 (2 hr.)	95 (7 hr.)	Decrease
7	80	100	80	60 (2 hr.)	80 (3 hr.)	Increase, then decrease
8	180	165	110	130 (1 hr.)	80 (6 hr.)	Decrease
9	95	70	90 (966m.)	90 (1 hr.)	90 (3 hr.)	Small decrease
10	125	165	105			Decrease
11	105	60	50	75 (1 hr.)	80 (2 hr.)	Decrease
12	195	160	110	170 (1 hr.)	130 (6 hr.)	Decrease
13	125	135	105	150		Small decrease
14	120	70		70 (1 hr.)	130 (2 hr.)	Decrease
16	95	115	90	50 (2.5 hr.)	110 (6.5 hr.)	Decrease
17	60	165	110 (970m.)*	100 (1 hr.)	95 (2 hr.)	Increase
19	60	150	135 (1000m.)*	150 (1.3 hr.)		Increase
"April 8"	200		193 (835m.)	105 (2.3 hr.)	130 (5 hr.)	Decrease
"April 14"	165		80*	60 (2.5 hr.)	120 (5 hr.)	Decrease
4 days later	210	517m.*	95 (0.5 hr.)	140 (2.5 hr.)	245 (4.5 hr.)	Decrease
"April 22"	105	30 (287m.)	70 (747m.)	95 (2 hr.)	50 (5 hr.)	Decrease

* Convulsions.

such an increase occurs (1). Vosburgh and Richards (2) were the first to demonstrate that epinephrin hastens clotting time.

The variable reaction in the clotting time as a result of exercise might possibly be accounted for by a difference in the amount of epinephrin set free although we have no definite data as to what this is during exercise. Cannon and Gray (3) were able to delay clotting by the injection of large amounts of epinephrin. However, such doses may be larger than that attained by physiological secretion resulting from exercise.

SUMMARY

The coagulation time of blood in twenty-one cats and one rabbit was determined before and at various times after exercise. The typical reaction was a decrease in coagulation time. A few animals showed an increase with or without a decrease following. The reduced coagulation time sometimes persisted for several hours.

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THE MECHANICAL EFFICIENCY OF THE MAMMALIAN HEART

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Studies of a denervated and perfused heart, such as functions in a heart-lung preparation, are always open to certain criticisms. The experimental elimination of numberless variable influences of the circulatory system must of course affect that part which has been isolated. On the other hand, it is unnecessary to enlarge upon the many advantages which the heart-lung preparation offers, in view of the extensive and pioneer investigations of Starling and his co-workers, carried out by means of this technique.

Information as to the utilization of energy by the heart is at the present time best obtained by means of the heart-lung preparation. It is possible to obtain the cardiac output (either by applying the Fick principle, or foreign gas absorption methods) and the pressure-head of an intact circulatory system, and so to calculate with fair accuracy the energy expenditure. A greater significance, however, can be attached to this knowledge if it is possible to correlate it with some understanding of the energy consumption.

The oxygen usage and mechanical efficiency of the heart were first studied by Rohde in 1910, and Rohde and Nagasaki in 1913, by means of a mammalian heart perfused with Ringer's solution. Studies were also made by Evans (1912) and Evans and Matsuoka (1915) on the heart-lung preparation perfused with blood. Their data were later recalculated by Evans (1918). The method employed by him was to determine the gaseous oxygen consumption of the entire preparation. In the present work we have used a similar method, but have substituted analysis of the blood, before and after its passage through the heart alone, for that of the respired gases. The possible advantages of this we will discuss later.

It was found necessary to modify somewhat the usual heart-lung preparation in order to make it suitable for our purposes. Cats were used as experimental animals and many difficulties were encountered at first, when the mixed defibrinated blood of more than one animal was allowed to circulate through the preparation, according to the usual technique as applied to dogs. Not only did pulmonary edema occur with great rapidity,

probably due to toxic products formed both by mixing the blood and by defibrinating (Lambert and Gremels, 1926), but even two cats provided an insufficient quantity of circulating fluid. Furthermore, these procedures must have altered the gaseous content and carrying capacity of the blood to some extent, since more or less hemolysis always took place; though just what this effect was, is not definitely known.

In the earlier work of Starling, hirudin had been used to prevent coagulation. In the present study, chiefly through the use of heparin

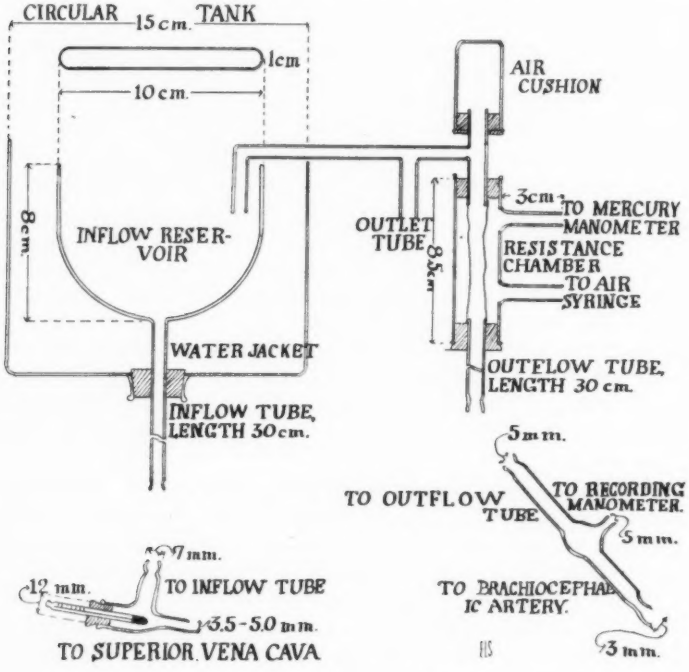

Fig. 1. Apparatus used for the single cat heart-lung preparation

(Howell, 1925) for the same purpose, it was finally possible for us to evolve a preparation demanding the use of but one cat, perfusing the heart and lungs with homogeneous blood. In this preparation we believe the blood was not altered in any manner affecting our results. The apparatus used and the details of the method are described below.

Method. The apparatus is the same in most particulars as that described by Knowlton and Starling (1912). The respective parts are arterial and venous cannula, a resistance chamber, an inflow reservoir, a

warming bath, and the necessary connecting tubing. Measurements of the apparatus as used for the cat heart-lung preparation are given in figure 1. The inner tube of the resistance chamber is made of rubber membrane, so that the resistance offered to the blood may come from the air pressure exerted upon it and not from the walls of the tube itself. This pressure can thus be transmitted to a mercury manometer. The inflow reservoir is flattened so as to expose a large surface of blood to the warming bath which surrounds it. The warming bath is maintained at the proper temperature by an ordinary electric light bulb immersed in it to the necessary depth, while a small stream of air bubbling through the water serves to keep it stirred. The significant blood temperature during the course of the experiments is read from the thermometer in the venous cannula. The temperature in the water bath needed to keep the blood temperature at the proper level varies somewhat, depending upon the rate of flow, the amount of blood in the inflow reservoir and other minor factors. As a rule, however, there need not be a difference of more than four or five degrees between the two.

As it seems essential for the success of the experiment to adhere closely to a uniform technique, we shall give the steps of the procedure in some detail.

Certain general precautions are taken. Following anesthetization (see appendix), the animal is kept warm, preferably by means of an automatically warmed cat-board. Great care is taken to avoid unnecessary hemorrhage, as every cubic centimeter of blood is usually needed. An artificial respiration apparatus is used which allows the lungs to deflate of their own elasticity. It also provides a uniform depth of respiration throughout the experiment. Care is taken to over-ventilate the lungs at no time, but merely to provide sufficient excursion to oxygenate the blood well. Over-ventilation is conducive to early pulmonary edema. While working in the open thorax, small quantities of warm isotonic saline are occasionally allowed to drop over the pericardium, in order to prevent drying and cooling.

The solution of heparin is first injected into a femoral vein. Forty milligrams were found to provide a sufficient excess to prevent coagulation in cats weighing from two to four kilograms, at the same time producing no discernible toxic effects and no hemolysis. As a precaution against inducing pulmonary edema, it is dissolved in no more than 5 cc. of isotonic saline. The heparin is then allowed to circulate through the blood stream while the vagi are isolated, the animal tracheotomized, and a cannula for bleeding inserted into one of the carotid arteries. Thirty-five to 60 cc. of blood are then withdrawn, and kept at body temperature until the heart-lung circulation has been established. (It is better not to attempt warming at all, than to overheat this blood, as over-heating is attended by the

production of toxic substances.) Following the bleeding it is advisable to apply a moderately tight bandage to the lower extremities and abdomen. Artificial respiration is then instituted and the vagi are severed, after which an incision is made directly down upon the mid-line of the sternum, and the thorax opened down the mid-line with bone forceps. The internal mammary vessels are severed between ligatures, and the chest held widely open with hooks. The fat and remnants of thymus surrounding the upper part of the pericardium and great vessels are carefully stripped off to permit cannulization, and the phrenic nerves avulsed. The azygos vein is then tied as it passes above the root of the right lung, and likewise the left subclavian artery, as it leaves the aorta. The branches of the brachiocephalic artery are ligated well above its bifurcation, and the superior vena cava directly opposite this point. Preparatory ligatures are placed around the inferior vena cava, and aorta just above the root of the left lung, but not tied.

The transition from the intact to the heart-lung circulation can now be made, and care is taken that the flow of blood through the heart be not interrupted during the procedure. The peripheral resistance in the resistance chamber is at first very low (30 to 40 mm. Hg) which seems to allow the heart to accommodate itself better to the first adjustment of the inflow.

Cannulization of the superior vena cava is first undertaken. After the cannula has been tied into the vein, it is connected with the inflow tube and adjusted so that it is evenly supported and not under any strain. Just sufficient saline is then allowed to flow in, by means of the inflow clamp, to fill completely both tube and cannula, while at the same time air bubbles are carefully expelled. When the saline level is just visible at the bottom of the venous reservoir, the cannula is closed with the thermometer, and the inflow clamp shut. The pinch-clamp used on the vessel can then be safely removed, and the reservoir filled with the previously collected blood.

The next step is the cannulization of the brachiocephalic artery. A retaining ligature is placed so that the mouth of the cannula will be held in the lumen of the aorta, just above the cusps of the aortic valve. This ligature is passed into a perforation made in the pericardium about one-quarter of an inch below and a little to the right of the point where the brachiocephalic artery leaves the aorta. It is then passed behind the artery, and directly out through the pericardium and fascia on its left side. A heavy pinch-clamp is then put on the artery at the site of the ligature, and the cannula inserted just below the bifurcation. The cannula is thrust down well into the aorta, and held there while a single knot is tied in the retaining ligature around it. The cannula is then slowly withdrawn so that the ligature can be drawn home around the groove and secured when the cannula has reached the proper position.

The aorta is now tied, and as much blood as possible quickly expressed from the abdomen into the heart. This will of course be pumped into the venous reservoir. As the blood is being expressed from the abdomen of the animal, the inflow clamp is slowly opened by an assistant so that from the two combined sources as constant an inflow as possible may be maintained. When the blood has been fully expressed from the abdomen, and the inflow clamp opened to the proper degree, the inferior vena-cava is tied, thereby completing the heart-lung circulation. The side arm of the arterial cannula is then attached to the manometer of the kymograph which is held at a low level, and the blood pressure record begun. The peripheral resistance should be slowly raised until the blood pressure has reached the desired height. There should be no delay before executing this last procedure as a blood pressure below 90 mm. will not maintain an adequate flow through the coronary vessels (Markwalder and Starling, 1913). Once the heart-lung circulation has been established and is working automatically, with the blood at the proper temperature, the subsequent procedures are a matter of choice.

Blood oxygen determinations. In order to determine the amount of oxygen consumed by the heart, samples of blood, approximately 15 cc. each, were taken from the inflow tube before, and from the pulmonary artery after, passage through the right heart. The blood from the inflow tube was presumably fully oxygenated, while that from the pulmonary artery was a mixture of the arterial blood with that which had flowed through the coronary circulation. The difference in oxygenation of the two bloods could thus be obtained, the product of this figure and the output of the heart, giving the oxygen consumption of the heart. Syringe needles connected to the collecting chambers were thrust directly into the inflow tube, and into the pulmonary artery just beyond the pulmonary valves, the latter needle being held in situ by an assistant during the procedure. Puncture of the right ventricle or the pulmonary conus for samples was found to produce arrythmias, and cannulization of the branches of the pulmonary artery was found to involve too many difficulties to be practical.

A series of experiments was first performed in which the blood was collected under oil, but this method incurred too large an error for the small quantities of gas extracted. The blood was then collected directly over mercury as described by Austin, Cullen, Hastings et al. (1922) and analyses carried out by the manometric method of Van Slyke and Neill (1924).

Each analysis was checked at least once. The mean deviation of all of the readings $\left(\text{m.d.} = \sqrt{\frac{\Sigma d^2}{n-1}}\right)$ was 1 mm.Hg on the manometer of the instrument, or of the order of 0.2 volume per cent oxygen.

During the collection of samples the cardiac output was frequently measured at the outlet tube above the venous reservoir, and the pressure recorded upon a revolving kymograph drum, in order to determine whether or not they were being maintained at constant levels. A rapid withdrawal of blood from the pulmonary artery caused marked alterations in the output and pressure, presumably through the change of inflow to the left ventricle. If the collection of samples, however, was extended over a period of about five minutes at a constant rate (controlled by stopcocks on the collecting chambers), the output and pressure remained constant. The discrepancy so produced in the outputs of the two ventricles amounted to 3 cc. per minute, or 4 per cent of the average output, which was considered by us as negligible in computing the work of the heart.

TABLE 1

DATE	OUTPUT	BLOOD PRESSURE	HEART RATE	HEART WEIGHT	O_2 CONTENT			O_2 DIFFERENCE	O_2 CONSUMPTION
					Arterial	Mixed venous and arterial	Saturated		
	liters/hr.	*mm.* Hg	*min.*	*grams*	*volumes per cent*	*volumes per cent*	*volumes per cent*	*volumes per cent*	*cc./gm./hr.*
7/16	5.35	127	238	13.1	13.0	11.9		1.1	4.5
7/17	9.25	144	272	14.6	11.8	10.8		1.0	6.7
7/19	4.10	141	270	17.6	11.0	9.1	11.4	1.9	4.2
7/20	3.02	150	281	13.4	12.4	10.5	13.5	1.9	4.3
7/28	1.62	83	156	10.1	8.2	6.1		2.1	3.3
8/3	4.14	130	231	10.2	12.7	11.4		1.3	5.1
8/4	3.55	103	252	9.4	11.7	10.2	12.7	1.5	5.9
9/2	6.42	96	235	10.3	11.2	10.3		0.9	5.6
9/4	5.02	96	250	13.2	9.7	8.2		1.5	5.5
9/6	4.02	149	210	11.6	9.2	7.3		1.9	6.4

In ten experiments there was no evidence of pulmonary edema, cardiac failure or any disturbance in rhythm, and the blood appeared at all times to be fully oxygenated within the duration of the experiment. In three of these experiments the oxygen-carrying capacity of the blood, as well as the content, was determined. The cardiac oxygen consumption in these experiments varied from 3.3 to 6.7 cc. per gram heart muscle[1] per hour. The output varied between 1.62 and 9.25 liters per hour, and was controlled principally by adjusting the inflow to the heart. The blood pressure varied between 83 and 150 mm. of mercury, and was controlled

[1] Wet weight. A source of error (probably not exceeding 5 per cent) was unavoidably introduced in weighing the hearts, although an attempt was made to dissect the great vessels and other non-contractile tissues away from the musculature as carefully as possible.

by means of the peripheral resistance chamber. There was considerable difference in the rates of the various denervated hearts (see table 1). The temperature of the blood at the inflow cannula was kept at 37.7°C., with an approximate variation of 0.3°C.

BLOOD-SUGAR DETERMINATIONS. Samples of blood for sugar determinations were drawn from the cat and later from the heart-lung preparation at different periods. A sample was drawn from a cannulized carotid artery immediately after the induction of the anesthesia, a second as soon as the heparin had time to circulate, a third after the completion of the operation, and a fourth and fifth simultaneously from the equilibrated circulation of the heart-lung. The fourth sample was taken as part of the

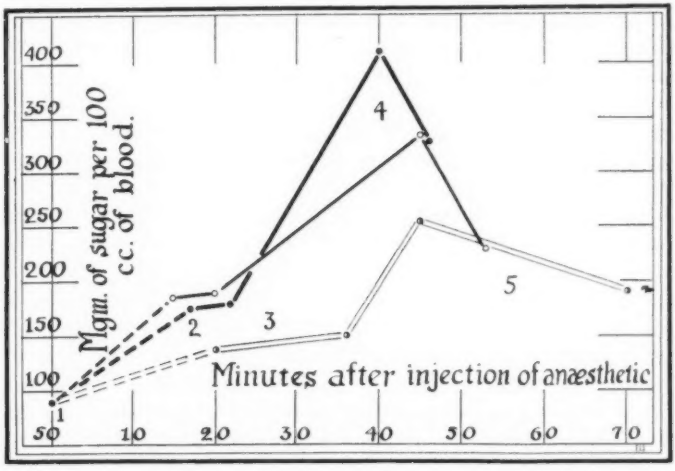

Fig. 2. Blood sugar during the course of three typical experiments. *1*, normal (Scott); *2*, immediately after induction of anesthesia; *3*, after heparin; *4*, after operation, circulation intact; *5*, heart-lung circulation.

blood used for venous oxygen content determination, and the fifth as part of the arterial blood simultaneously collected. Analyses were made immediately after drawing the samples, in order to prevent the loss of any sugar by glycolysis. The method of analysis used was that of Shaffer and Hartman as modified by Duggan and Scott (1926).

According to the work of Scott (1914) and others, pain or excitement causes an immediate hyperglycemia in the cat, so that Scott secured his normal samples by decapitation. He gives 70 mgm. per 100 cc. of blood as the average, which we have used as a basis for point *1* on the curves representing the blood sugar variation through the course of typical experiments (fig. 2). As stated elsewhere in this communication, the

anesthetic used causes an average rise of 150 per cent in the blood sugar. The anti-coagulant, heparin, does not appear to affect the blood sugar. A test showed it to be itself non-reducing, and we may attribute the small rise after its injection to the further action of the anesthetic. Analysis of the blood drawn after the completion of the operative procedure showed a marked hyperglycemia, in one case rising to 410 mgm. per 100 cc. of blood. This again may be explained as due partly to the added action of the anesthetic and partly to the various stimuli to which the animal was subjected during the course of the operation. Point *5* on the curve, representing the content of the blood circulating in the completed preparation, shows a decided drop in the blood sugar. This was anticipated, it being a mixture of that blood just described as having the most marked hyperglycemia (point *4*), and that set aside in a flask (see method for preparation of the heart-lung) which was even found to be subject to a slight fall in sugar content due to glycolysis.

Sugar determinations on the samples of venous and arterial blood drawn for the previously described gas analysis, and measurement of the cardiac output, enable us to estimate the rate of sugar disappearance per hour. Between the blood drawn from the inflow tube (arterial), and blood drawn simultaneously from the pulmonary artery (venous plus arterial), there is an average difference of 5.2 mgm. per 100 cc. As the error of analysis is of the order of 2 mgm., any given experiment means little. An average for a series of experiments, however, will give the order of magnitude of sugar disappearance. On a basis of ten experiments the rate of sugar disappearance per gram of heart muscle (wet weight) per hour, was found to be of the order of 20 mgm.

Our figures for the disappearance of sugar are considerably higher than those of most investigators. Clark (1916) gives a summary in tabular form of the work done prior to his. The results vary from 0.7 to 14.9 mgm. per gram of heart weight per hour. The cat, however, has rarely been selected for this type of experimentation, because the susceptibility of this animal to pulmonary edema renders it less adaptable. Furthermore, to the best of our knowledge all previous work has been done on preparations which were allowed to run over a comparatively long period of time, with sugar determinations being made at appropriate intervals to study the disappearance. By the method just described the samples were drawn immediately after the equilibration of the preparation, which we believe more closely approximates the normal conditions. The blood has been circulating through the single organ for a much shorter period, and presumably the insulin supply has not as yet been appreciably affected. Furthermore, a heart acting for a prolonged period of time under the conditions of the heart-lung preparation undergoes degenerative changes, and very probably a decreasing amount of sugar is taken from the circulation.

There is reason to believe that sugar disappearance is the result of more than simple oxidation. Various forms of condensation probably occur at the same time (Clark, 1916, 1917). For this reason we have not attempted a correlation between the oxygen consumption and the sugar disappearance.

Discussion. The principal component of cardiac work is the product of the blood output and the pressure to which it is raised. The work expended in imparting velocity to the blood stream (accelerating) is sometimes assumed to be insignificantly small, but according to Evans, in certain conditions such as relatively large outputs, or narrowed aortic orifices, the acceleration factor may amount to from 16 to 20 per cent of

TABLE 2

DATE	TOTAL WORK	ACCELERATION COMPONENT OF WORK	O_2 CONSUMPTION	EFFICIENCY
	kilogram-meters	*per cent*	*cc./gm./hr.*	*per cent*
7/16	10.48	1.7	4.5	11
7/17	20.99	3.5	6.7	12
7/19	8.89	0.9	4.2	6
7/20	6.90	0.4	4.3	6
7/28	2.01	0.2	3.3	12
8/3	8.24	1.0	5.1	16
8/4	6.01	2.0	5.9	17
9/2	9.68	3.4	5.6	17
9/4	7.45	1.9	5.5	5
9/6	9.15	0.8	6.4	5

the total work of the heart. We have calculated the total work and that fraction represented by this factor, for our experiments, by means of Evans' revised formula (1918).

$$W = \frac{7\,QR}{6} + \frac{w(VC)^2}{g\,E^2}$$

This formula allows for alteration in the relative duration of systole in the acceleration component. The mean pressure developed by the right ventricle is taken as one-sixth that of the left, according to Führer and Starling (1913). The smallest diameter of the aortic cannula used by us was 2.5 mm.; the velocity of the blood passing through the cannula being calculated from the output per second and the cross-sectional area of the cannula.

[2] W = work done, in kilogram-meters; Q = volume of blood expelled in liters; w = weight of blood, in kilos; R = arterial resistance, in meters of blood (i.e., mm. Hg $\times$ 0.013); V = velocity of blood at root of aorta, in m. per sec.; g = acceleration due to gravity = 9.8 m. per sec.; C = duration of the cardiac cycle; E = duration of the period of expulsion (assumed for our purpose as one-half of C).

The mechanical efficiency of the heart, or the ratio of its energy expenditure to its energy utilization, was calculated from the work done, and from the oxygen consumption on the same basis employed by Evans, that one cubic centimeter of oxygen is equivalent to 2.06 kilogram-meters of work. The figures for the cardiac work and efficiency are given in table 2.

Evans has shown that the oxygen consumption of a heart acting under relatively normal conditions is proportional both to the amount of peripheral resistance and the output which it must contend with; or expressed differently, to the amount of work imposed upon it, within physiological limits. The denervated rate being practically constant at body temperature, his results were not affected by it. Changes in heart rate would of course have a direct effect upon the consumption of oxygen.

Abnormal conditions of the cardiac muscle, as in states of heart failure, or when acting under the influence of depressing drugs have been shown to produce marked lowering of efficiency.

Our experimental procedure precluded drawing more than one set of samples for each experiment, so that the effect of changing conditions upon the individual hearts could not be studied. However, an attempt was made to establish a relationship between the various hearts and their efficiencies, through the conditions imposed upon them. No definite relationships could be found between the work and efficiency of the hearts and any of the working conditions or any combination of conditions. There appears to be a marked individual variation in efficiency. It is possible that this variation may have been due to undetermined experimental factors, such as changes in blood pH, lactic acid content or others, but as all observations were made immediately after the transition from the intact circulation, we do not believe very marked changes would have had time to occur. It seems justifiable to us to presuppose intrinsic differences in the individual hearts, possibly related to their age, or differences in structure, in order to account for the differences in efficiency. It was, however, impossible for us to obtain significant respiratory quotients for the acting heart muscle. The so-called "steady state" had presumably not been reached at the time the blood samples were drawn, accounting for a variable carbon-dioxide combining power and dissociation curve. Under these circumstances, we cannot definitely say that some variation in the consumption of oxygen was not due to differences in the rate of reconversion of lactic acid to glycogen.

SUMMARY

1. A modification of the heart-lung preparation is described which requires the use of only one animal, and which permits the circulation of autogenous blood.

2. The oxygen consumption of hearts performing different amounts of work was from 3.3 to 6.7 cc. per gram per hour. The mechanical efficiency of these hearts was found to vary from 5 to 17 per cent.

3. Attempts to establish a relationship between the factors of work and the oxygen consumption of the various hearts were unsuccessful. It is thought that differences in condition may account for the relative difference in efficiency.

4. The rate of sugar disappearance from the blood passing through the hearts was of the order of 20 mgm. per 100 cc. blood, per gram per hour.

We wish to thank Prof. E. L. Scott and Mr. H. F. Pierce for their interest and help in this study. We are indebted to Mr. Louis Dotti for the blood sugar determinations.

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APPENDIX. The selection of an anesthetic for animal experimentation is always a problem that requires serious consideration. Aside from its general convenience, obviously an anesthetic should be selected which interferes as little as possible with the normal physiological processes, so that the effects of the anesthetic may not be confused with those of other factors being investigated.

Complete surgical anesthesia has been induced by us in over two hundred cats, for this and other studies, with a mixture of two parts of trichlorisopropyl alcohol (isopral), and one part of methylpropylcarbinolurethane (hedonal) by weight, warmed to liquefaction. Some of the effects of this mixture have already been described by Lambert (1926), but his investigations were carried out on a considerably smaller series of animals than in the present communication.

Dosage: On a basis of 46 experiments we have found the average dosage for cats

to be 0.154 cc. of the pure mixture per kilogram of body weight (injected intraperitoneally with a tuberculin syringe), and to vary from 0.13 to 0.20 cc. per kilo. We have particularly noted that large muscular animals usually require the maximum dosage for this series, and sometimes even more. For cats of 2.0 to 3.2 kgm., 0.15 cc. per kilo is usually sufficient. There was a mortality of two in two hundred, and two more were saved, probably by the institution of artificial respiration. Lethal doses appear to cause death through the production of respiratory failure.

Stages and duration of the anesthesia: 1. Somnolence—This stage occurs within a very short time after the intraperitoneal injections of the anesthetic, about two minutes being the average.

2. Ataxia—Follows somnolence and usually occurs in within four to six minutes after the injection. During this period the sneezing reflex may be excited.

3. Excitement—This period usually is accompanied by hyper-active reflexes and there may be considerable excitement if the animal is stimulated.

4. Anesthesia—Occurs after an average of nine minutes. The onset may occur as late as fifteen minutes when a single dose is sufficient to induce anesthesia. The animal may exhibit rhythmic movements, such as a bicycle riding movement of the legs. When the nictitating membranes are found to be partially covering the eyeballs, the animal is usually fully anesthetized.

During ten experiments, the animals were on the operating board for times ranging from three to seven hours, after which they were killed. A few cats were anesthetized, merely for observation, and kept properly warmed until they came out of anesthesia. When the dose given was sufficient for complete surgical anesthesia the animals remained anesthetized for about ten hours, after which they passed into a deep sleep and then awoke in a weakened condition, which lasted for about twenty-four hours more.

Effect of the anesthetic on respiration and circulation: Records of the rate and amplitude of respiration, of the blood pressure and of the heart beat were taken of seven cats. The only operative procedure was that of cannulizing the carotid artery. The respiratory movements were followed by means of a large balloon bandaged on the abdomen, and connected to a tambour which recorded the movements on a moving kymograph paper. The times over which these records were taken varied from three to seven hours.

The results obtained showed a small variability. On the whole, the rate and amplitude of the respiratory movements were normal. The blood pressure remained at an average level of about 130 mm. of mercury, and varied from 110 to 145 mm. falling below 110 mm. in only one experiment. The rate and nature of the heart beat were normal, the rate varying directly with the blood pressure. The variations of the blood pressure and heart rate were partly attributed to the original operative procedure and stimuli that the animal was subjected to in adjusting the apparatus.

Effect of the anesthetic on the blood sugar: According to Scott (1914) the normal blood sugar of the cat is 70 mgm. per 100 cc. of blood. We have found the blood sugar of 12 anesthetized cats to be of an average of 184 mgm. per 100 cc. of blood and to vary from 144 to 234 mgm. The procedure consisted of anesthetizing the animal and drawing a sample of blood from a cannulized carotid artery. The method of analysis used was the Shaffer-Hartman method for 1 cc. of blood, as modified by Duggan and Scott (1926). The average time for eleven experiments for the drawing of the blood sample, after the injection of the anesthetic, was 18 minutes and varied between 11 and 41 minutes. The time for one experiment was two hours. There is some indication of a correlation between the time of drawing the sample and the height of the blood sugar.

The hyperglycemia caused by the use of this anesthetic was expected. Much work has been done to show that anesthetics and narcotics uniformly cause a rise in the blood sugar. According to Page (1923) and later Edwards and Page (1924), iso-amylethylbarbituric acid (amytal) is the only anesthetic supposed not to have such an action. Macleod confirms this (1926).

The isopral and hedonal were very kindly supplied by the Winthrop Chemical Company.

INFLUENCE OF EXPERIMENTAL HYPOTHYROIDISM UPON GASTRIC SECRETION

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The undertaking of this problem is a continuation of the researches of Hardt (1916) and Truesdell (1926) suggested to the writers by Doctor Carlson.

EXPERIMENTAL. Four Pavlov pouch dogs were used, two of which were carried through the complete series. The experiments were run after a day of starvation and were followed by a day of rest during which time food was given ad libitum. Each experiment consisted of collecting and testing one sample of the basic (continuous) secretion and six samples of the digestive juice collected hourly after giving a secretory meal of 200 grams fat-free sheep heart, 50 cc. water, and 50 cc. milk thoroughly mixed. Calcium lactate and carbonate in doses of 1.5 gram per kilo body weight per day were given orally to control the tetany following complete thyroparathyroidectomy (Luckhardt and Goldberg). Prior to thyroparathyroidectomy experiments were run to determine the effect of the calcium (1.5 gram per kilo per day) upon gastric secretion. An electric heater was used to raise the body temperature in order to see what part the increased body temperature, observed during thyroid feeding, played in the depression of gastric secretion.

RESULTS. Of the four dogs one was put on thyroid feeding and three were thyroidectomized. Two of these died in tetany and later the fourth dog was thyroidectomized and observed.

Thyroid feeding. In view of the fact that the dose of thyroid used by Hardt and by Truesdell was reasonably large, smaller and more physiological amounts were tried, the dose used being 0.05 gram U. S. P. desiccated thyroid per kilo per day. This dose was sufficient, however, to raise the body temperature about one degree at the end of the sixth hour after feeding. Consequently a temperature control was run using as a source of heat an ordinary electric heater. The results of thyroid feeding and artificial heating are summarized in table 1.

Thyroidectomy. After determination of the normal gastric secretory response to the standard secretory meal, and of the secretion under calcium medication, three of the dogs were thyroidectomized. Two of these were

lost in tetany and the third was carried through the series of experiments summarized in table 2.

Table 1 also shows the results of thyroidectomy upon the dog which had previously been used for thyroid feeding. It is interesting to note that this

TABLE 1

Tabular review of experiments performed on 10 kilo Pavlov pouch dog to show effect of thyroid feeding, thyroidectomy, calcium medication, and raising body temperature upon gastric secretion as tested over a six-hour period after the ingestion of a standard secretory meal. The volume and free and total acidities are recorded

CONDITION OF EXPERIMENT	NUMBER OF EXPERIMENTS	VOLUME			FREE ACIDITY			TOTAL ACIDITY		
		Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
Normal	6	93	49	66	0.51	0.44	0.48	0.54	0.48	0.52
Thyroid feeding	6	41	25	33	0.46	0.37	0.40	0.49	0.42	0.45
Control	5	59	37	47	0.47	0.40	0.43	0.51	0.44	0.46
Temperature control	2	68	51	59	0.43	0.40	0.42	0.47	0.45	0.46
Calcium control	6	68	38	55	0.45	0.33	0.41	0.50	0.35	0.45
Post-thyroidectomy	6	100	76	86	0.47	0.46	0.46	0.50	0.48	0.49
Thyroid feeding	5	45	33	36	0.40	0.35	0.37	0.43	0.37	0.40

TABLE 2

Tabular review of experiments performed on 10 kilo Pavlov pouch dog to show effect of calcium medication, thyroidectomy, and subsequent thyroid feeding upon gastric secretion as tested over a six-hour period after the ingestion of a standard secretory meal. The volume and free and total acidities are recorded

CONDITION OF EXPERIMENT	NUMBER OF EXPERIMENTS	VOLUME			FREE ACIDITY			TOTAL ACIDITY		
		Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
Normal	5	22	17	20	0.36	0.29	0.32	0.41	0.35	0.37
Calcium control	3	25	15	20	0.30	0.27	0.28	0.36	0.34	0.35
Post-thyroidectomy	4	39	26	32	0.43	0.35	0.39	0.47	0.41	0.44
Thyroid feeding (1 gram / day)	1			26			0.36			0.42
Thyroid feeding (2 grams / day)	2	15	14	15	0.20	0.16	0.18	0.29	0.28	0.29
Control	5	42	21	29	0.46	0.32	0.39	0.47	0.35	0.41

dog (table one) was thyroidectomized early in August and in spite of the very warm weather (the animal frequently registered a rectal temperature of 102 to 104 and at times panted) which was conducive to dehydration and subsequent depression of gastric secretion, there was obtained a very appreciable rise in the total volume of gastric juice secreted.

SUMMARY

The results of thyroid feeding confirm those of Hardt and of Truesdell, i.e., a daily dose of 0.05 gram per kilo body weight was capable of depressing gastric secretion both in amount and acidity. This can hardly be attributed to the rise of one degree of body temperature accompanying thyroid feeding, since elevation of one degree of body temperature by external heat, although not strictly comparable, did not give the same results.

In both dogs reported here thyroidectomy resulted in a decided increase in volume of gastric juice and in a less marked but nevertheless demonstrable increase in acidity. The calcium salts in one dog were found to have no effect on gastric secretion and in the other a slight depressing effect. The results of thyroid feeding and thyroidectomy, however, far outweighed the calcium factor. Thyroid feeding subsequent to thyroidectomy, was capable of again depressing gastric secretion, which came back to its previous level when the thyroid feeding was stopped.

In brief, we have carried the work of Hardt and of Truesdell one step farther and have shown not only that experimental hyperthyroidism depresses gastric secretion but also that experimental hypothyroidism increases gastric secretion.

It is with pleasure that we acknowledge our indebtedness to Doctor Carlson and Doctor Luckhardt for their guidance and suggestions in this investigation. Thanks are also due Mr. Mandel Spivek for his skilful assistance.

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IS THYROIDECTOMY THE PRIMARY CAUSE OF THE ARRESTED DEVELOPMENT OF SKELETAL MUSCLE IN CRETIN SHEEP?

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During the spring of 1926 a comparative study of striated muscle in normal and thyroidectomised sheep was made by the writer (1) to determine whether or not definite structural changes in the fibers were present which would explain the development of the characteristic pot-belly and general physical weakness in the cretins. It was found that no degeneration of the fibers takes place. Following extirpation of the thyroid gland, however, the normal growth of the muscle is retarded and, in the thyroidectomised animal, the ratio of nucleus to cytoplasm is more nearly like that in the young animal than in the control twin.

Upon reporting that following thyroidectomy there was a marked decrease in the development of the muscle-fibers of the sheep, the question was raised whether this might not be due rather to lack of exercise incident upon thyroidectomy than directly assignable to absence of the thyroid hormone. Liddell (2) determined that the cretin animal constantly fails to exhibit the same activity as the normal sheep. May we attribute to this lethargy the non-development of the muscular system?

The obvious and direct method of answering this question would be to restrict the activity of a normal sheep by confining him to a pen. In the course of another investigation made by Liddell and Simpson (3) the pedometer readings showed that control sheep kept in close quarters got rid of their excess energy by jumping and racing around the small inclosure to such an extent as to render that method of inactivation futile. On the other hand, it is just as impracticable to exercise a cretin. Following thyroidectomy the tissues show such pathological changes, notably hyaline degeneration of the thoracic and abdominal aortae and pulmonary arteries, that strenuous exercise would probably be fatal in a short time (4).

In the summer of 1926 our experimental flock became infected with round worms and tape-worms. Those control animals which survived the double parasitic invasion are dwarfed to such an extent that they are little larger than their thyroidectomised twins. The "normal" sheep became as lethargic as their cretin twins after the establishment of the parasites and

exercised as little as did the operated animals. So on one hand were sheep rendered inactive by parasites; on the other, animals lethargic from thyroidectomy and parasites, the only difference being the presence of the thyroid gland in one group and its absence in the other. If the lack of development of the muscle-fiber is due primarily to thyroidectomy, we would expect to find the muscle of the controls normal in size, while if lack of exercise is the dominant factor in the non-development of the muscular system, we should find the fibers of the controls approximately equal in cross-section area to those of the cretins.

Sutherland Simpson (5) determined the influence of thyroidectomy on the growth of sheep and goats, using body-weight as an indicator. From

TABLE 1 (5)

Normal growth of control and cretin lambs from the third to the seventh month

ANIMAL	AGE AT THYROIDECTOMY	WEIGHT IN POUNDS AT 3 MONTHS, AUGUST 8, 1920	WEIGHT IN POUNDS AT 7 MONTHS, DECEMBER 11, 1920
	days		
8♂ norm		37	64
8♀ op	42	34	37
9♂ norm		41	72
9♀ op	42	33.5	38

TABLE 2

Growth of control and cretin lambs from the third to the seventh month, showing the same stunting from thyroidectomy and parasites

ANIMAL	AGE AT THYROIDECTOMY	WEIGHT IN POUNDS AT 3 MONTHS, AUGUST 4, 1926	WEIGHT IN POUNDS AT 7 MONTHS, DECEMBER 11, 1926
	weeks		
T norm., 1926		41	49½
T op., 1926	4	45	36½
H norm., 1926		48	44½
H op., 1926	4	43	46½

table 1 may be seen the increase in weight which takes place in normal and cretin lambs from the third to the seventh month.

In marked contrast to the values in table 1 are the weights of the sheep infested with worms. At the age of seven months one "normal" is actually lighter than its cretin sister (table 2). Sheep T control 1926 and H control 1926 are far below usual weight due to the parasites while the cretins suffer doubly from thyroidectomy and worms. In appearance, however, the "normal" animal is quite different from the cretin, the former preserving his alertness in contrast to the typical stupid look of the thyroidectomised animal.

Slips were removed from the pectoralis major muscle of the two pairs of lambs, T norm. and T op 1926 and H norm. and H op 1926. H op and T op were thyroidectomised at four weeks of age and, in addition, all the animals were infested with round and tape-worms. A histological study of the muscle-structure shows no degenerative changes. The cross-section areas of the fibers were calculated for each animal (table 3). It is evident that from July 6, 1926 to December 14, 1926 the muscle of H norm. increased only 22 per cent. These five months would normally represent a period of very great growth in the young animal. On the other hand the fibers of T norm. increased 177.7 per cent in the same length of time. We must conclude either that T norm. had fewer parasites than H norm. or

TABLE 3

The cross-section area of the muscle-fibers from the cretin lambs is comparable to that of the controls dwarfed by parasites

ANIMAL	DATE	X-SECTION AREA IN SQUARE MICRONS	NO AREAS COUNTED	PER CENT
H norm., 1926	July 6, 1926	726.78	20	100.0
	December 14, 1926	886.69	40	122.0
H op., 1926	July 1, 1926	566.62	60	100.0
	December 14, 1926	563.26	60	99.6
T norm., 1926	July 7, 1926	326.85	20	100.0
	December 14, 1926	907.9	40	277.7
T op., 1926	June 25, 1926	666.9	20	100.0
	December 14, 1926	651.44	60	97.6

that the animal had a greater resistance to their deleterious effects. The fibers of H op, a lamb thyroidectomised at the age of four weeks, decreased 0.4 per cent from July to December while the muscle of T op was reduced in cross-section area 2.4 per cent.

From the investigation into growth of muscle following thyroidectomy referred to earlier (1), the value determined as normal enlargement of the muscle-fibers was 282 per cent in one year, while following thyroidectomy the increase in size was 14 per cent.

We may conclude that after thyroidectomy the non-development of the muscle-fibers is largely due to reduced activity. A heavy parasitic invasion is capable of bringing about partial cessation of skeletal muscle growth in proportion as it curtails exercise.

SUMMARY

1. In a previous study of skeletal muscle in normal and cretin sheep it was found that, after removal of the thyroid gland, the muscle fibers practically cease to grow. Is this non-development the direct effect of the athyroid condition or is it indirectly due to lack of exercise following thyroidectomy?

2. In control sheep early stunted by parasites and as lethargic as their cretin twins, the cross-section area of the muscle-fibers is comparable to that of the thyroidectomised animals.

3. The lack of development of the striated muscle in cretin sheep is due rather to lethargy than directly attributable to the athyroid condition.

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